Modification of Glassy Carbon Electrodes with Diazonium Cation Terminated Films: "Sticky Surfaces"

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Abstract

This thesis described the modification of glassy carbon (GC) electrodes with aminophenyl (AP) films via in situ reduction of aminobenzene diazonium ions. The characterisation of the AP modified GC was conducted electrochemically by oxidation of the AP functionalities in acidic aqueous conditions. Ferricyanide and ruthenium hexamine redox probes were also used to investigate the blocking properties of the AP films. Before electrochemical oxidation of the AP functionalities, AP films were shown to have a nett positive charge at pH 7. After electrochemical oxidation in protic conditions, the film was either neutral or negatively charged.

The preparation of diazonium cation terminated surface, which is termed 'sticky surface', by reaction of the AP modified electrodes with $NaNO_2$ in acidic condition, was investigated and the sticky surface was electrochemically characterised. More than one species was formed in the reaction of the AP film with $NaNO_2$. The reactions of sticky surface with aniline, citrate- and thiol-capped gold nanoparticles (Au-nps) were also studied. Spontaneous reaction of sticky surface with thiol-capped Au-nps had been achieved, and suggested that the reaction leads to the formation of Au–C bonds, via the loss of nitrogen. However, for the reaction of the sticky surface with citrate-capped Au-nps, it was unclear whether covalent bonding had been achieved. The reason for this was due to the possibility of an electrostatic interaction between the negatively charged citrate-capped Au-nps and the positively charged sticky surface.

The stability of the sticky surface in acidic aqueous conditions was studied electrochemically and by reaction with thiol-capped Au-nps. It was found that the dizonium cations on the sticky surface are not stable over one hour in aqueous acidic

conditions, or even in low temperature. The electro-catalytic activity of the thiol-capped Au-nps attached to the GC electrode via sticky surface towards the oxidation of ascorbic acid was briefly examined, and the surface was found to catalyse the oxidation reaction.

Abbreviations

μA	Microampere
ACN	Acetonitrile
AFM	Atomic force microscope
AP	Aminophenyl
Ar	Aryl ring
Au-nps	Gold nanoparticles
μC	Microcoulomb
CNT	Carbon nanotube
CV	Cyclic Voltammogram
DMSO	Dimethylsulfoxide
E	Potential
ΔE_p	CV oxidation and reduction peak potential separation
$E_{½}$	Half wave potential
$E_{p,a}$	CV anodic peak potential
$E_{p,c}$	CV cathodic peak potential
EtOH	Ethanol
GC	Glassy carbon
i	Current
KCl	Potassium chloride
mM	Millimol L^{-1}
MQ	Milli-Q/millipore
OCP	Open circuit potential
PB	Phosphate buffer
PBS	Phosphate buffer saline
PPF	Pyrolyzed photoresist film
Q	Charge

s	Seconds
SAM	Self-assembled monolayer
SCE	Saturated calomel electrode
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TOABr	Tetraoctylammonium bromide
UV	Ultraviolet
ν	scan rate
V	Volt
V_f	Final volume
XPS	X-ray photoelectron spectroscopy

Chapter 1. Introduction

Surface modification can result in surfaces having new and specific functionalities that are useful in numerous applications such as, biosensors,[1-4] catalysis,[5] fuel cells[6-8] and molecular electronics.[9-12] Carbon-based electrodes are widely employed for use in these applications, because they have many attractive properties: low cost compared to precious metal electrodes; chemical inertness in most electrolyte solutions; electrical conductivity; mechanical stability; and a moderately wide operating potential window (typically – 1 V to + 1 V in aqueous electrolytes).[13-15] The modification of carbon surfaces via reduction of aryldiazonium salts to achieve a covalent bond between the surface and the modifier has been achieved.[16-18] In this thesis, the modification of carbon surface with a diazonium cation terminated surface is investigated. Glassy carbon (GC) is chosen as the substrate for surface modification.

1.1. Glassy Carbon

GC is generally fabricated from polymeric resins such as polyacrylonitrile or phenol/formaldehyde polymers through a series of heat treatments (usually under pressure) between 1000 and 3000 ^{o}C that resulted in a hard, conductive carbon material that is impermeable to solution.[19-23] GC may also be made from a reactive polymeric precursor at relatively low temperature (~700 ^{o}C), which permits "doping" with various heteroatoms in the polymer, as well as the final product, including halogens, silicon, and metal catalysts.[24, 25]

GC has been extensively studied since it was first produced in the early 1960s. The structure of GC is still the subject of debate, due to a lack of direct experimental data.

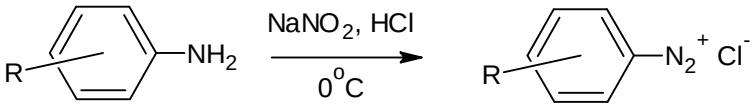
Presently, GC is described as network of randomly oriented graphitic carbon or ribbons.[19, 20, 23] Graphitic carbon consists of basal and edge plane. Graphite edge plane sites are known to be more reactive towards chemical reaction than basal plane sites.[14, 24, 26, 27] The low reactivity of the graphite basal plane is due to its low density of electronic states and to the lack of functional groups and adsorption sites.[28-30] Electron-transfer is generally believed to be faster at the edge plane than at basal plane[28, 30] of graphite. Basal planes have a low local density of states near the Fermi energy, whereas edges have much higher local densities of states due to σ bonds and/or terminating impurity groups.[31] Therefore, there is a higher chance that electrons will be available for redox reactions at the edges rather than on basal planes. However, recent research by Unwin and co-workers[32] suggests that this may not be correct. More work is required to understand electron-transfer processes at graphite surfaces.

GC is generally pre-treated either electrochemically or mechanically to obtain a reproducible electrode surface. Electrochemical pre-treatment is usually performed by cyclic scanning over a wide potential range. Mechanical polishing is generally performed by polishing the GC electrodes on a micro-fabric cloth with ~ 1 micron, or finer, alumina slurry or diamond paste, followed by sonication in an appropriate solvent to remove the alumina (or diamond paste). This mechanical pre-treatment was used in this thesis to prepare GC surfaces prior to modification.

1.2. Aryldiazonium Salts

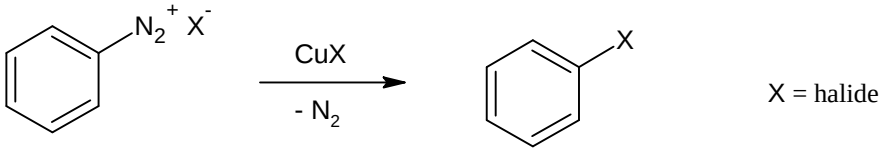
Aryldiazonium salts are organic compounds that have a common formula, $R\text{-}N_2^+X^-$, where R is an aryl group and X is an inorganic or organic anion such as Cl^- or BF_4^-. The process of forming aryldiazonium salts is called diazotization, which was discovered by Peter Griess in 1858.[33] Usually, diazotization is carried out by addition of nitrous acid to

aromatic amines at about 0 ^{o}C (Scheme 1.1). The nitrous acid is generated in situ from sodium nitrite and mineral acid. Diazotization can also be carried out in acetonitrile in the presence of *tert*-butyl nitrite.

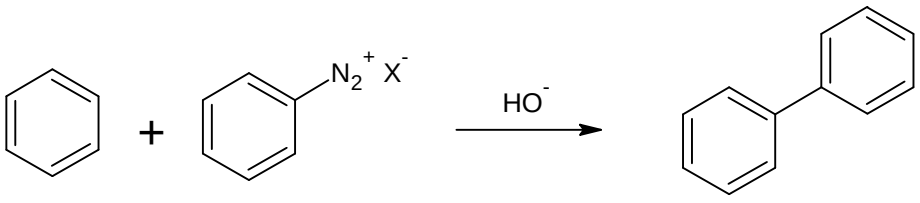


Scheme 1.1. Diazotization of aromatic amines.

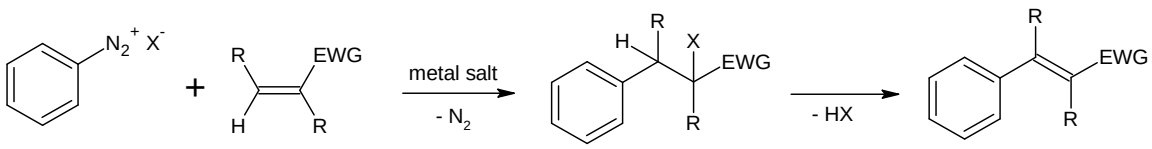
Aryldiazonium salts can undergo the process of dediazoniation, where the diazo group is displaced by releasing the stable dinitrogen molecule. Several well-known methods have been used to carry out dediazoniation such as, the Sandmeyer reaction,[34-36] where the diazo group is replaced by halides with copper salt as a catalyst (Scheme 1.2), Gomberg-Bachmann reaction,[37, 38] where diazonium salts is used to couple aryl groups (Scheme 1.3) and Meerwein arylation,[39, 40] which involves the addition of aryldiazonium salts to an electron deficient alkene (Scheme 1.4). Besides these methods, reduction of aryldiazonium salts by a substrate (electrode) in the modification of surfaces also leads to the dediazoniation process.[41, 42]



Scheme 1.2. An example of Sandmeyer reaction.

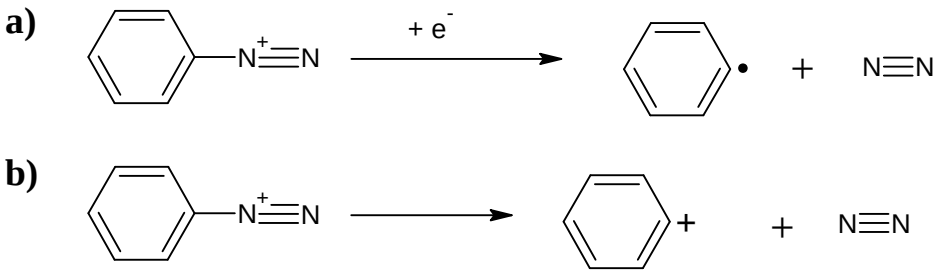


Scheme 1.3. An example of Gomberg-Bachmann reaction.



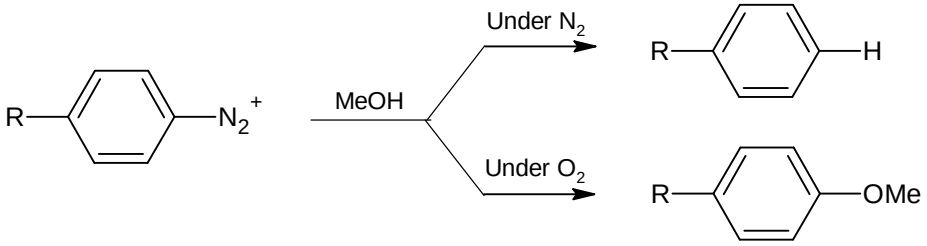
Scheme 1.4. An example of Meerwein arylation reaction. EWG is an electron withdrawing group.

There are two common dediazoniation pathways, homolytic (Scheme 1.5a) and heterolytic (Scheme 1.5b). The homolytic pathway requires an electron transfer from a reducing agent to the diazonium cation, which results in the formation of an aryl radical and dinitrogen molecule. The electron can be obtained electrochemically,[43] photochemically[44, 45] or by a chemical reducing agent.[33, 34, 46-49] The heterolytic pathway proceeds via a thermal decomposition by the loss of dinitrogen and formation of an aryl cation.[33, 45, 50, 51]



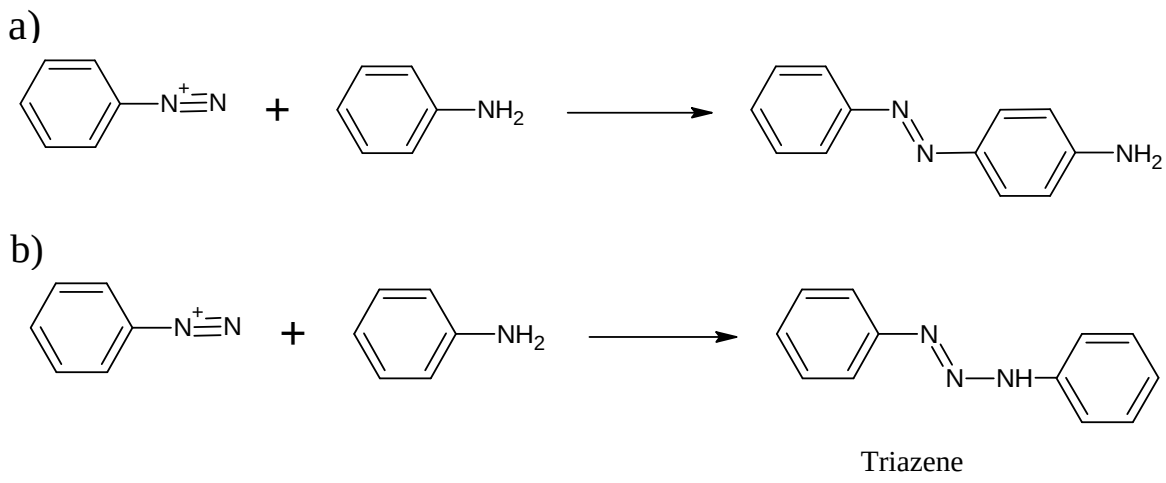
Scheme 1.5. Dediazoniation of benzenediazonium cation: a) homolytic and b) heterolytic.

Reactions of diazonium ions are very sensitive to reaction conditions, a slight change of the experimental conditions often results in different product. For example, reaction of unsubstituted benzenediazonium ion with nitrobenzene in DMSO proceeds mainly via *meta* substitution to 3-nitrobiphenyl; on the other hand, the reaction of 4-nitrobenzenediazonium ion with nitrobenzene leads to the formation of *ortho*- or *para*-substitued products (2,4′-and 4,4′-dinitrobiphenyl).[33] Furthermore, Bunnett and co-workers[52, 53] have found that the dediazoniation of aryldiazonium ions in methanol results in the formation of the corresponding hydrocarbon under nitrogen atmosphere, whereas in the presence of oxygen the corresponding methoxy substituted compound is formed (Scheme 1.6).



Scheme 1.6. Dediazoniation of benzenediazonium cation in methanol under different atmospheres.

Besides dediazoniation, aryldiazonium salts can also undergo a coupling reaction (azo coupling). It is a typical electrophilic aromatic substitution, where the diazonium compound acts as an electrophile that couples to a nucleophile, usually an aromatic hydrocarbon (Scheme 1.7). The coupling component is required to have a hydroxyl or an amino group substituent to increase its nucleophilicity as the diazo components are relatively weak electrophiles.[33] Either C-coupling can occur (Scheme 1.7a) or N-coupling which gives triazenes (Scheme 1.7b).[33, 54]



Scheme 1.7. Example of coupling reaction: a) C-coupling; b) N-coupling.

In azo coupling reaction involving an arylamine, there is always a possibility that the C-coupling is competing with the N-coupling. Usually under acidic condition, C-azo coupling is preferred over the N-azo coupling. Moreover, modification in the reaction conditions can also change the outcome of the product. For example, the coupling reaction of 4-methoxy-benzenediazonium tetrafluoroborate in dry acetonitrile with 3-

methylaniline predominantly resulted in the C-coupling product. However, on addition of *tert*-butylammonium chloride, the product was predominantly triazene.[33]

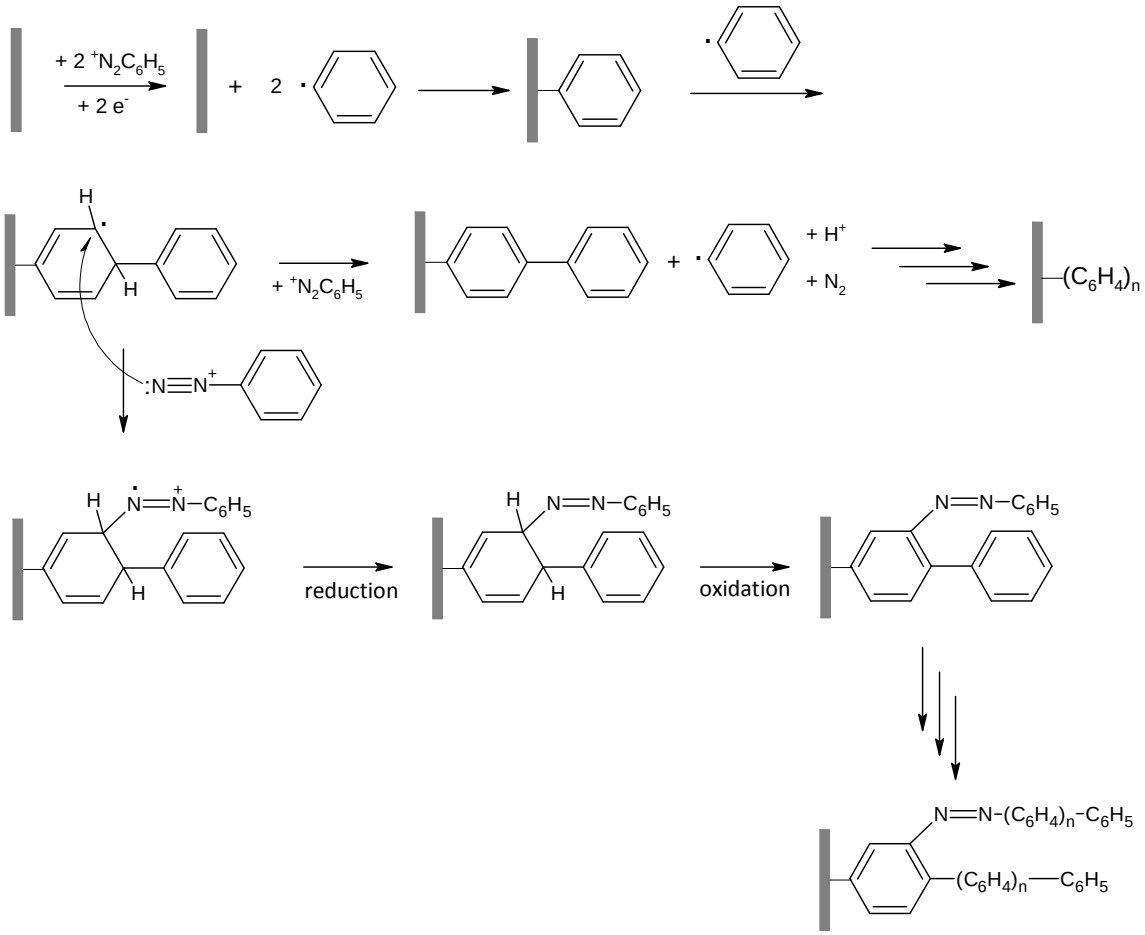
1.3. Surface Modification via Reduction of Aryldiazonium Salts

Generation of an organic radical in solution near a surface can lead to attack of the radical on the surface, resulting in the formation of a covalently bonded film at the surface.[16, 55-59] The most common method used to generate radicals is the reduction of aryldiazonium salts. Grafting from diazonium salt solution can be carried out either electrochemically[10, 17, 18, 60-71] or non-electrochemically.[72-81] In the electrochemical method, the aryldiazonium cation is reduced by application of an external potential, leading, via homolytic dediazoniation, to an aryl radical that grafts onto the electrode surface through a covalent bond (Scheme 1.9). Electrografting of a wide range of aryldiazonium salts has been shown to proceed at carbon,[16, 42, 62, 82, 83] metal[55, 58, 84-86] and semiconductor surfaces.[60, 61]

Scheme 1.9. Proposed mechanism for modification of surfaces via reduction of aryldiazonium salts.

Two non-electrochemical approaches have also been developed. In one approach, the diazonium cation is reduced directly by the substrate at open circuit potential (OCP),[74, 77, 78] hence the reaction is "spontaneous modification". The second approach involves using an added chemical reducing agent such as iron powder[87, 88] or hypophosphorous acid (H_3PO_2).[72, 81, 89, 90] Spontaneous modification has been demonstrated on carbon,[73, 74, 80, 89] metal[73, 74, 76-79] and semiconductor surfaces.[9, 91]

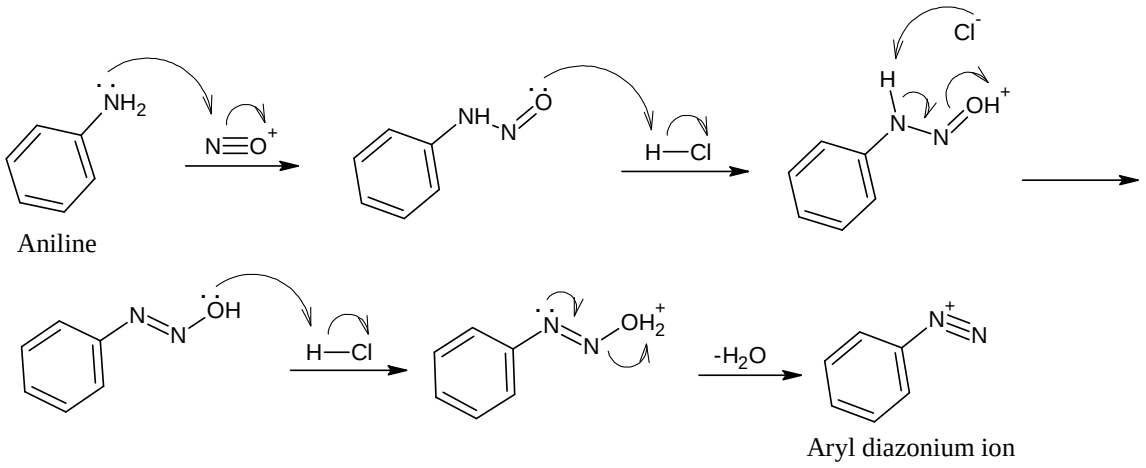
Both electrochemical and non-electrochemical grafting methods give very similar film properties and structure although film grafted spontaneously is generally thinner and forms more slowly than film that is grafted electrochemically. Formation of multilayer films has been observed at GC, PPF, Si and metal surfaces.[18, 42, 60, 65, 83, 92-94] Downard and co-workers[83, 94] have shown that the film thickness depends on the modification potential. More negative applied potentials result in thicker film, which indicates that film growth requires effective electron transfer from the substrate to diazonium cation in solution.[94] Moreover, the concentration of diazonium salts used and the modification time also control the film thickness especially in spontaneous grafting.[74, 95] Incorporation of azo groups in the formation of multilayer film structure is possible when aryl radicals attack the aryl groups that are already attached to the surface (Scheme 1.10).[96]



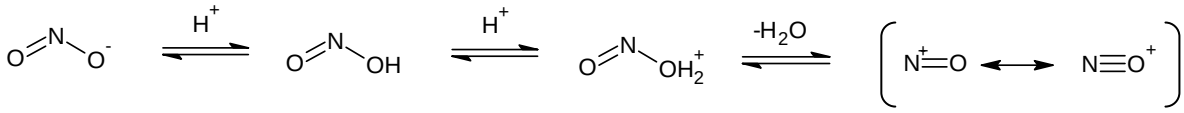
Scheme 1.10. Mechanism proposed by Pinson and co-workers[96] for the formation of multilayers and incorporation of azo bonds into the diazonium derived film.

1.4. Diazonium-Cation Terminated Films: Sticky Surface

"Sticky surface" is a term used to describe a surface containing a terminated diazonium functionality. The diazonium functionality is usually obtained from conversion of amine groups by addition of nitrosonium ion (Scheme 1.11). Nitrosonium tetrafluoroborate ($NOBF_4$), *tert*-butylnitrite and isoamylnitrite are usually used as the diazotising agent in acetonitrile,[7, 88, 97, 98] while in aqueous condition, nitrosonium ion can be obtained by reaction of sodium nitrate ($NaNO_2$) with acid (Scheme 1.12).

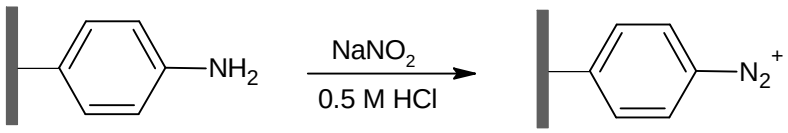


Scheme 1.11. Reaction of nitrosonium ion with aniline to form a diazonium ion.



Scheme 1.12. Reaction of nitrite with acid to form a nitrosonium ion.

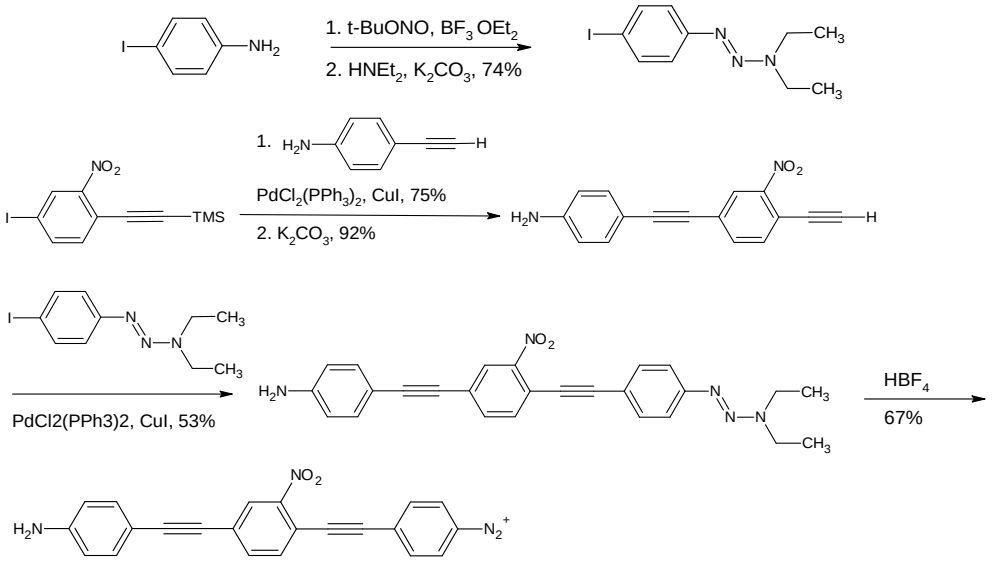
The most common method of producing sticky surface is by conversion of aminophenyl (AP) grafted film into diazonium terminated film through the use of $NaNO_2$ in HCl (Scheme 1.13).[88, 99-104]



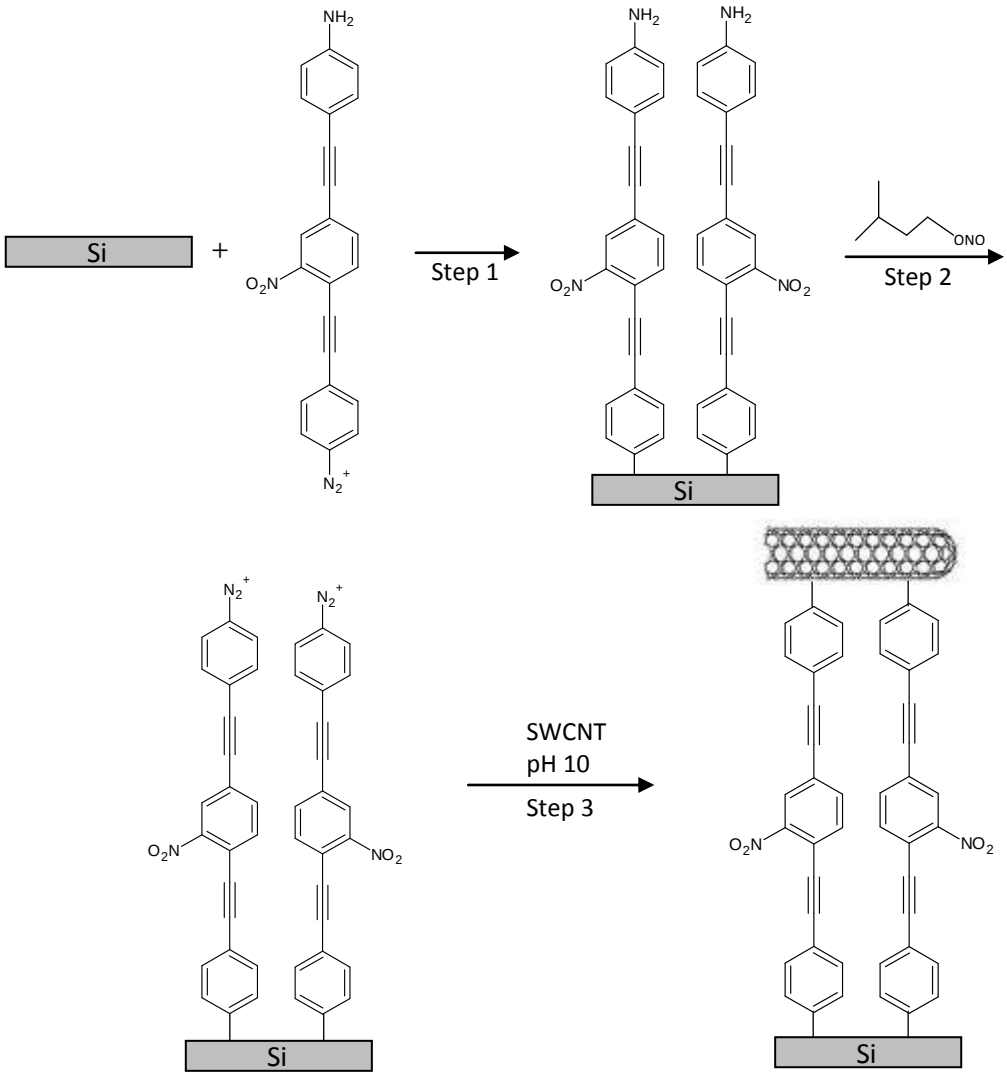
Scheme 1.13. The formation of sticky surface via AP film.

The purpose of a sticky surface is to achieve adhesion (covalent grafting) between a solid support (electrode) and a target species. The sticky surface is expected to react spontaneously with strongly nucleophilic compounds, carbonaceous materials which are able to provide electron transfer, or metallic compounds that are strong reducing agents. For a less reactive material, an external source of electrons is required for the reaction to proceed. Physical activation (thermal or UV irradiation) or chemical activation (electrochemical or reducing agent) is usually employed to initiate the reaction of the sticky surface with the target species.

Sticky surfaces have been shown to react with a range of species. Tour and co-workers[97, 98] have covalently immobilised single wall carbon nanotubes (SWCNTs) onto a silicon surface via reaction with a sticky surface. The procedure began with synthesis of a molecule bearing an α-triazene on one end of a linear conjugated oligo-phenylene ethynylene (OPE) backbone, and an aniline group at the other end (Scheme 1.14). The triazene group was then in situ converted into aryldiazonium using HBF_4, followed by spontaneous grafting to silicon surface (Scheme 1.15, step 1). This grafted OPE bearing an aniline functionality was then converted into the corresponding aryldiazonium cation by reaction with isoamylnitrite in acetonitrile (step 2).



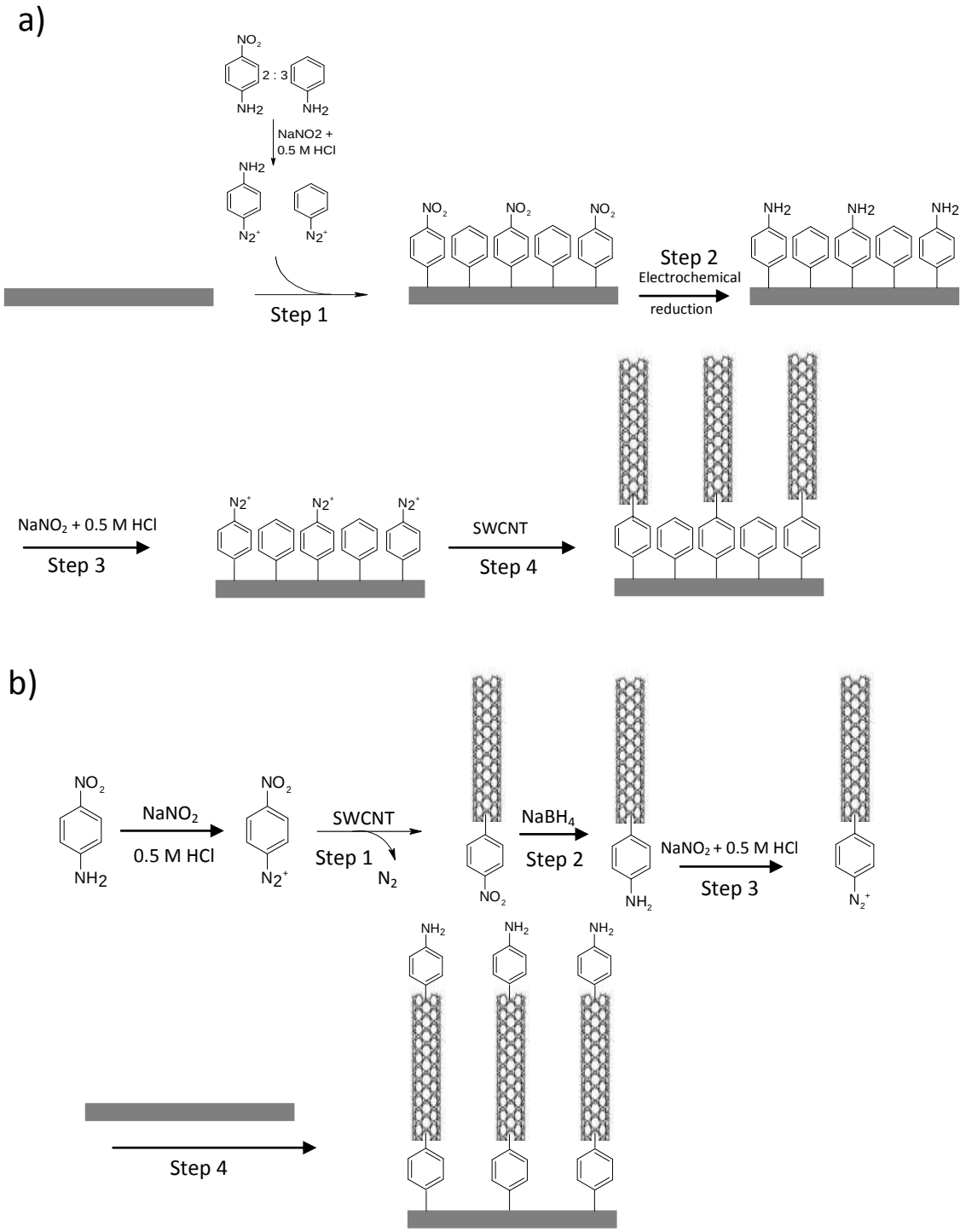
Scheme 1.14. Reaction conditions to produce oligo-phenylene ethynylene aryldiazonium salt.



Scheme 1.15. Attachment of carbon nanotubes to silicon via aryldiazonium chemistry.

Another simpler method of anchoring SWCNTs onto a surface via diazonium reaction was introduced by Ferri and co-workers.[100] Two routes to anchoring were investigated. In the first route, the surface was first modified by mixed phenyl and nitro groups (Scheme 1.16a, step 1). The nitro groups were then electrochemically converted to amine groups (step 2), which were then converted to diazonium functionalities by addition of $NaNO_2$/HCl (step 3). The SWCNTs were reacted with the diazonium terminated surface (step 4). In the alternative route, the SWCNTs were first functionalised with *p*-nitrobenzene groups (Scheme 1.16b, step 1). AP groups were formed by reduction of the nitro groups with $NaBH_4$ (step 2). The amines were then converted to diazonium ions, by

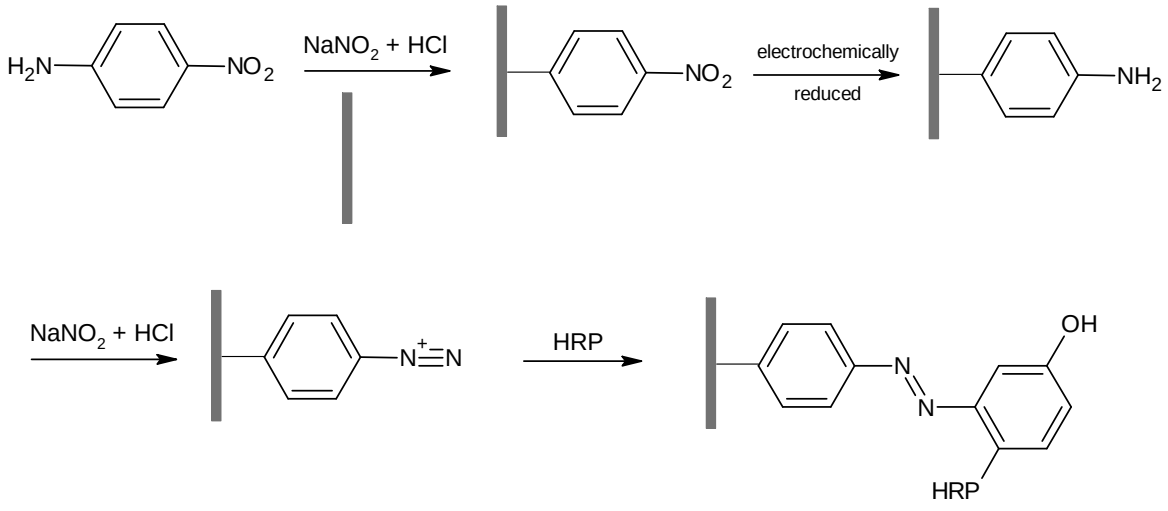
addition of $NaNO_2$/HCl (step 3), which covalently bind the SWCNTs to the surface (step 4).



Scheme 1.16. Schematic representation of SWCNT assembly on surfaces: a) immobilisation of unfuctionalised SWCNTs on surface modified by mixed of phenyl/diazonium groups; b) Self-assembly of diazonium-functionalised SWCNTs on a bare surface.

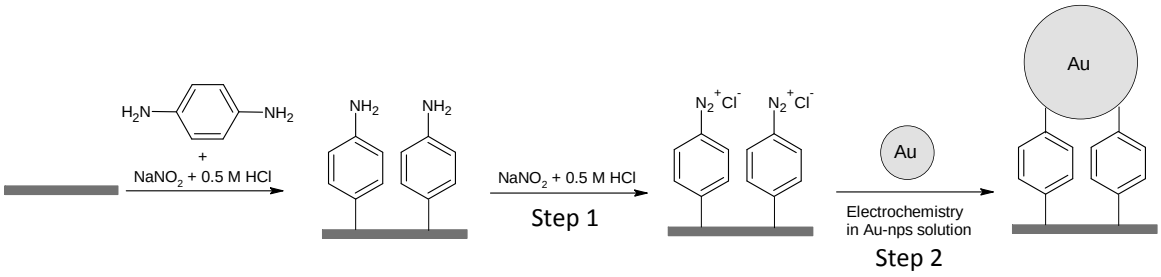
Similar attachment of double-walled CNTs using these two routes has also been investigated by Pinson and co-workers,[101] where ethylenedianiline was used as a linker instead of *p*-nitroaniline. Viel and co-workers[88] have also successfully attached multi-walled CNTs via diazonium functionalised surfaces. Besides MWCNTs, they have also shown that a nitrogenated organic compound, graphene sheets and copper nanoparticles react spontaneously with the sticky surface. Spontaneous attachment of silicon nanoparticles onto graphite flakes has also been reported by Brousse and co-workers.[7]

Recently, Viel and co-workers[99] have spontaneously immobilised glucose oxidase and DNA to a solid support via the use of sticky surface. Other biological materials, such as the enzyme horseradish peroxidise (HRP), have also been successfully immobilised on solid supports via sticky surface.[3, 103, 105] However, Radi and co-workers[3] proposed that attachment of HRP occurred via azo coupling of the diazonium ions, through phenolic, imidazole, or amino side chain of the HRP (Scheme 1.17), rather than by dediazoniation.



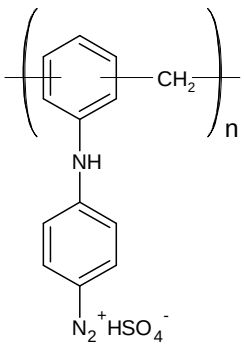
Scheme 1.17. Immobilisation of HRP to surface modified with diazonium functionalities via azo coupling reaction.

The attachment of gold nanoparticles (Au-nps) through electrochemical activation of the sticky surface has been reported by Gooding and co-workers.[104] In this method, the sticky surface was prepared by using $NaNO_2$/HCl to convert the electrografted amine functionalities into diazonium functionalities (Scheme 1.18, step 1). The diazonium modified electrode was then electrochemically reduced in Au-nps solution to achieve covalent grafting (step 2). It was proposed that aryl radicals formed during the electroreduction step covalently bind to the Au-nps.

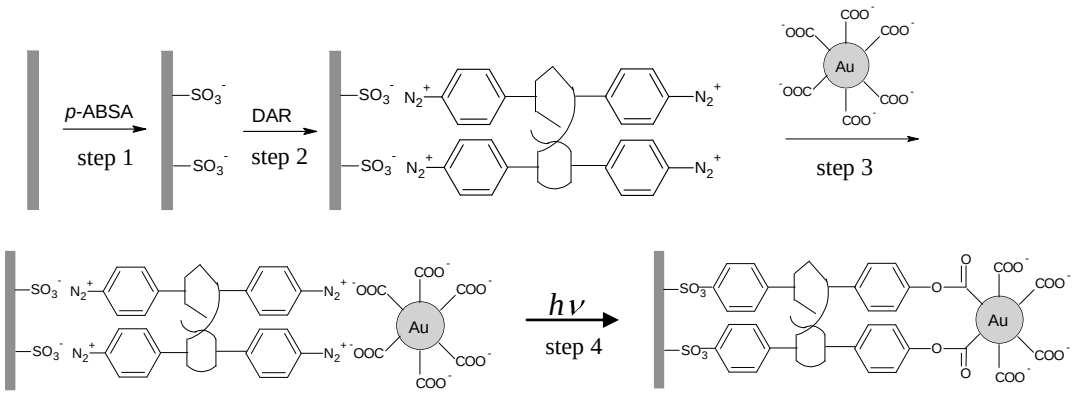


Scheme 1.18. Covalent attachment of Au-nps on solid supports via electrochemical activation of sticky surface.

Sun and co-workers[106] have covalently attached Au-nps onto surfaces, via diazo-resins (DAR, Scheme 1.19), by UV activation of the diazonium functionalities. In this method, the surface was first electrochemically modified with *p*-aminobenzene sulfonic acid (*p*-ABSA) to yield a sulfonic-functionalised film (Scheme 1.20, step 1). The sulfonic-acid functionalised surface was then reacted with DAR (step 2) and citrate-capped Au-nps (step 3). The fabricated films were then exposed to UV light to obtain covalent attachment (step 4).



Scheme 1.19. Chemical structure of DAR.



Scheme 1.20. Covalent attachment, by UV activation, of Au-nps on surface functionalised with DAR.

Since use of the sticky surface approach is a relatively new development, very little is known about the properties of the sticky surface and factors that influence the attachment of substrates onto the sticky surface. Accordingly, the work carried out in this thesis aims to investigate the properties of the sticky surface and its reactions with solution species.

1.5. Applications of Surface Modification

The potential applications of modified surfaces with specific functionality include sensors (chemical and biological)[4, 70, 107-113] and molecular electronics.[11, 12, 101, 114] Typically a sensor consists of an electrode (signal transducer), recognition element and a chemical layer that enables the immobilisation of the recognition species on the electrode surface (Figure 1.1).[111] The recognition species must have high selectivity toward the target analyte and is effectively immobilized onto the electrode surface to ensure long-term performance of the sensor.

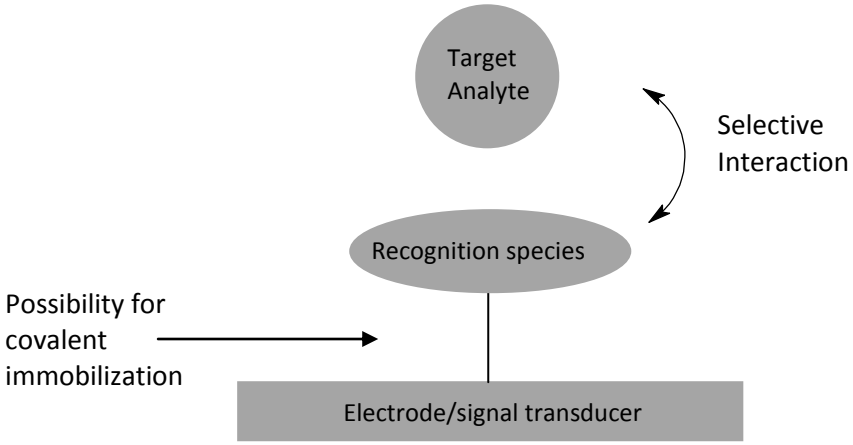
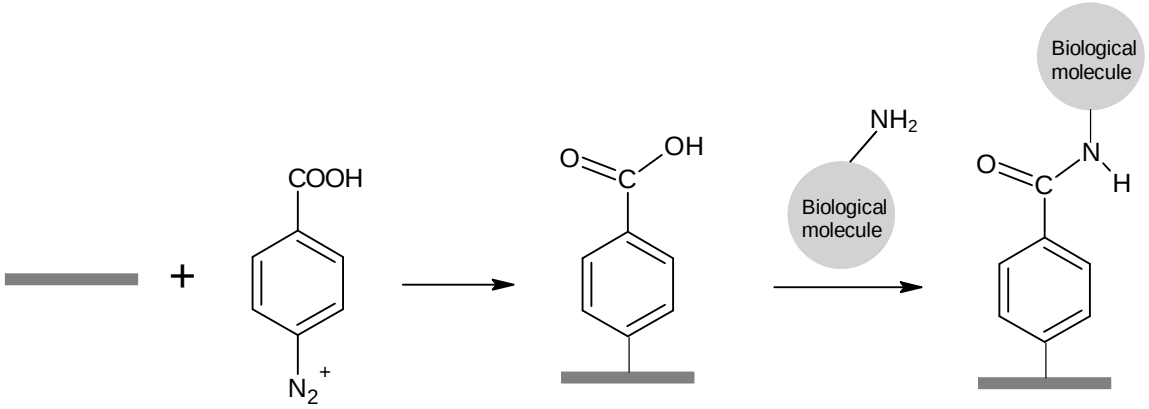


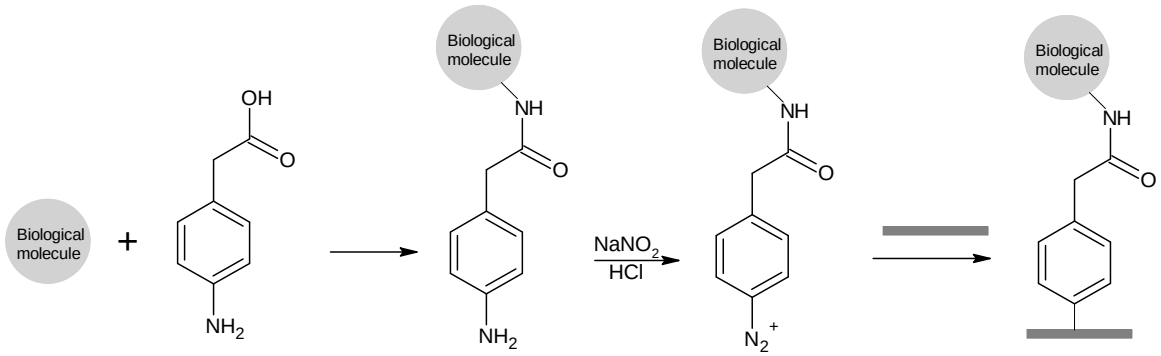
Figure 1.1. General schematic for a sensor.

Three general approaches based on aryldiazonium chemistry have been used to covalently immobilise biological molecules on surfaces for biosensor applications. In the first approach, the surface is modified with amine or carboxylic acid functionalities via diazonium ions, the biomolecules are then covalently immobilized on the surface via the formation of amide bonds between the surface's amine/carboxylic acid groups and the biomolecules' carboxylic acid/amine groups (Scheme 1.21).[1, 2, 115] Coupling agent, such as carbodiimide, is usually added to the reaction to promote the formation of the amide bond. This process may create some side products that can affect the activity of the biomolecules.



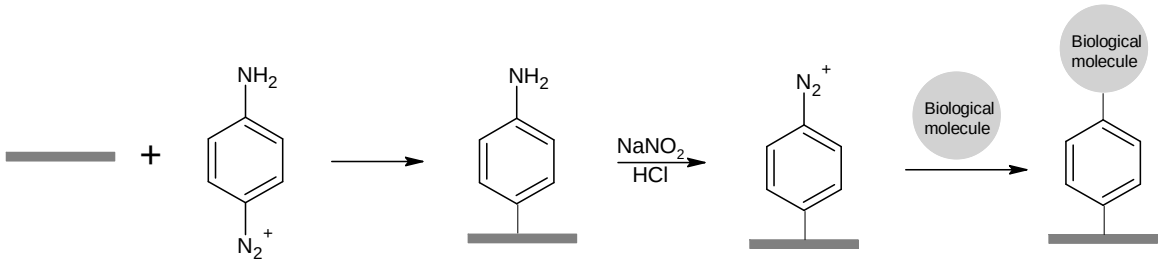
Scheme 1.21. Schematic representation of immobilisation of biological molecule on a surface via surface bound carboxylic functionalities.

In another approach, the biomolecules are first modified with *p*-carboxymethylaniline, which is then diazotised into aryl diazonium ions prior to the immobilisation on the surface (Scheme 1.22).[112, 115-117] The limitation of this approach is that chemical modification of biomolecules may damage the biological activity of the molecules and it is also a time consuming process. Therefore, it is of interest to develop more versatile methods to provide a strong and stable binding of biomolecules.



Scheme 1.22. Schematic representation of immobilisation of biological molecule on surface via modified biological molecule with diazonium functionalities.

As mentioned earlier, covalent immobilisation of biological molecules through surface modified diazonium functionalities (Scheme 1.23) has been investigated by Viel and co-workers.[99] This method appears to provide cleaner products and is less time consuming than the alternative method.



Scheme 1.23. Schematic representation of immobilisation of biological molecule on surface via sticky surface.

Chemical sensor fabrication based on diazonium chemistry has also been developed. For example, screen-printed carbon electrodes modified with *p*-carboxyphenyl groups have

been used for Cu(II)[118] and uranium[119] detection. Moreover, pH sensors have also been fabricated, via the use of diazonium cation, to obtain a pH-sensitive hydroquinone functionalised surface.[120]

Surface grafting using aryldiazonium cations also has potential applications in electronic devices. Current microelectronic devices are fabricated on silicon surfaces with copper interconnects (lines or trenches if the connection is horizontal and vias if vertical) between the transistors. As microelectronic devices have decreased exponentially in size over the past years, the need for a replacement of the copper is necessary. CNTs are one of the possible molecules that can be used to replace copper connections. Covalent attachment of CNTs to silicon trenches has been achieved using aryl diazonium chemistry.[121] The fabrication of molecular electronic junctions based on diazonium salts has also been investigated by McCreery and co-workers.[122]

1.6. Aims

The aims of this project are:

- To gain a better understanding of *p*-aminophenyl film grafted onto GC via the in situ reduction of aryldiazonium salts;
- To prepare a sticky-surface from an AP modified film and to study the sticky-surface electrochemically;
- To investigate the reactivity of sticky-surface towards gold nanoparticles and aniline.
- To briefly investigate the catalytic properties of thiol-capped gold nanoparticles towards the oxidation of ascorbic acid.

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Chapter 2. Experimental

2.1. Introduction

This chapter outlines the general experimental methods and materials used throughout the work of this thesis. Experimental information relevant to work in a specific chapter can be found in the experimental section of that chapter.

2.2. Synthesis and General Solutions

All aqueous solutions were prepared using Millipore (MQ) water, resistivity > 18 MΩ cm.

2.2.1. Phosphate Buffer Solution

Phosphate buffer saline (PBS) of 0.05 M, was prepared by dissolving 0.328 g of sodium di-hydrogen phosphate (NaH_2PO_4), 0.412 g of di-sodium hydrogen phosphate (Na_2HPO_4) and 5.85 g NaCl in MQ water (final volume, V_f = 100 mL) to give pH 7.

Phosphate buffer (PB) of 0.2 M (pH 7.0), was prepared by dissolving 0.978 g of NaH_2PO_4 and 1.97 g of Na_2HPO_4 in MQ water (V_f = 100 mL).

2.2.2. Gold Nanoparticles Preparation

2.2.2.1. Citrate-Capped Gold Nanoparticles

Citrate-capped gold nanoparticle suspension was prepared using a previously reported procedure.[1] All glassware was washed with aqua regia solution (3 : 1, HCl : HNO_3) followed by rinsing with MQ water before use. Tetrachloroauric acid ($HAuCl_4$, 500 mL, 1

mM) solution was brought to the boil with stirring. Sodium citrate (50 mL, 38.8 mM) solution was rapidly added to the $HAuCl_4$ solution. The concentration of sodium citrate used was varied to obtain different size nanoparticles.[2] For the 17 nm diameter nanoparticles, 39 mM of sodium citrate was used, while for 26 nm diameter size nanoparticles, 20 mM of sodium citrate was used. After adding citrate the solution was boiled for 10 minutes with stirring. The colour of the solution turned from pale yellow to colourless and finally to red. The solution was then removed from the heat and stirring was continued for another 15 minutes. The solution was cooled to room temperature and filtered through a membrane filter (Millipore, 0.22 μm). The gold nanoparticle solution was stored in the dark. The average particle diameter was determined from scanning electron microscopy using Image J software. For the smaller nanoparticles, measurement of 272 particles gave an average diameter of 17 ± 2 nm, while for the larger nanoparticles, measurement of 258 particles gave an average diameter of 26 ± 5 nm.

2.2.2.2. Thiol-Capped Gold Nanoparticles

Thiol-capped gold nanoparticle suspension was prepared according to the method of Brust and co-workers.[3] An aqueous solution of $HAuCl_4$ (30 mL, 30 mM) was mixed with solution of tetraoctylammonium bromide in toluene (TOABr, 80 mL, 50 mM). The two-phase mixture was vigorously stirred until all the $AuCl_4^-$ was transferred into the organic layer (the aqueous layer changed from yellow to colourless and the organic layer became red) and 1-decanethiol (200 μL) was then added to the mixture. A freshly prepared aqueous solution of sodium borohydride ($NaBH_4$, 25 mL, 0.4 M) was added dropwise to the mixture with vigorous stirring. The solution was stirred for a further 3 hours. The colour of the solution turned from red to yellow and then colourless and finally to dark brown. The organic phase was separated and evaporated to approximately 10 mL on a

rotary evaporator. Ethanol (400 mL) was then added to the solution and the mixture was kept at –18 ^{o}C overnight. The Au-nps formed a dark brown precipitate and were filtered and washed with ethanol. The nanoparticles were then re-dispersed in toluene (100 mL) and stored in the dark. From the transmission electron microscopy (TEM) images (Figure 2.1), the nanoparticles have diameters < 3 nm. TEM images were performed by David Anderson (Chemistry Department, University of Canterbury) on a Philips CM-200 TEM.

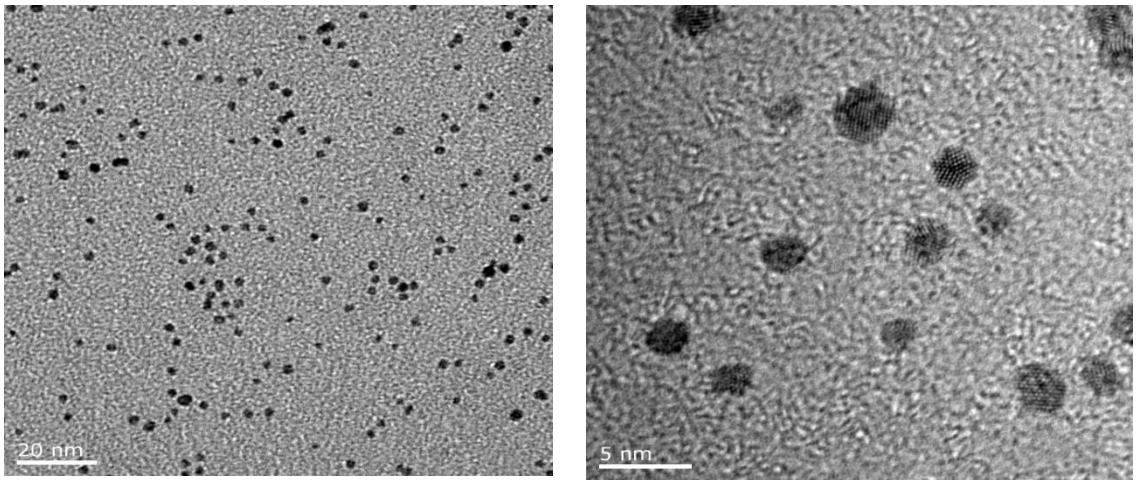


Figure 2.1. TEM images of the thiol-capped gold nanoparticles.

2.3. Electrochemical Methods

2.3.1. Instrument and Software

Electrochemical measurements were performed using an Ecochemie Autolab PGSTAT302 potentiostat/galvanostat interfaced to PC computer system and controlled by Autolab General Purpose Electrochemical Systerm (GPES) software version 4.9.

2.3.2. Electrodes

Glassy carbon (GC) electrodes were cleaned before any electrochemistry was carried out. The GC was hand polished with a slurry of 1 micron alumina on a piece of micro-fabric

cloth until there were no visible markings or scratches. The GC electrode was ultrasonicated (Elmasonic S 30 H sonicator) in MQ water for 5 minutes to ensure the elimination of the alumina from the surface. This procedure was repeated after each set of experiments.

There are two different types of GC surfaces used in this work (Figure 2.2), GC disks (area = 0.071 cm^2) sealed in Teflon and GC plates (working area = 0.289 cm^2, which is defined by the O-ring used) for different cell setups as described in the section below.

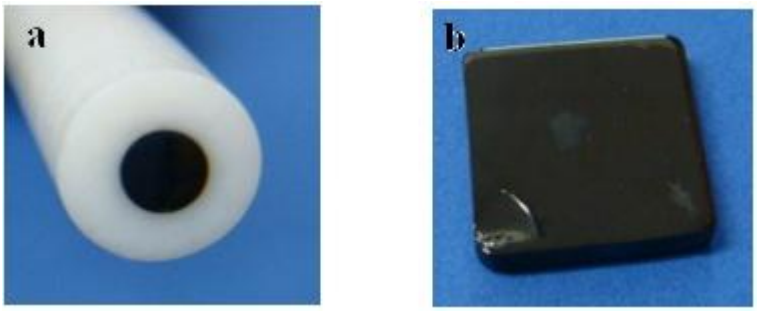


Figure 2.2. Photographs of the different GC surfaces being used: a) disk and b) plate.

2.3.3. Cell Setup

The electrochemical cells were washed in a 10 % HNO_3 acid bath after each set of experiments. The cells were thoroughly rinsed with MQ water prior to use. A three-electrode cell was used for experiments with the GC as working electrode, platinum as auxiliary electrode and a saturated KCl calomel electrode (SCE) as reference electrode.

Two types of electrochemical cell were used to carry out experiments, either a standard pear shaped glass cell (Figure 2.3a) or a pear shaped glass cell with a hole in the base (Figure 2.3b). For the standard glass cell a GC disk working electrode was used, whereas for the cell with a hole in the base, a GC plate was positioned between the metal base holder and the glass cell. A Kalrez O-ring was placed between the GC surface and the glass cell to provide a seal to avoid leakage of solution. A strip of copper was placed

between the GC and the metal base holder to maintain electrical contact, and the setup was then secured by four metal springs attached to the four corners of the cell.

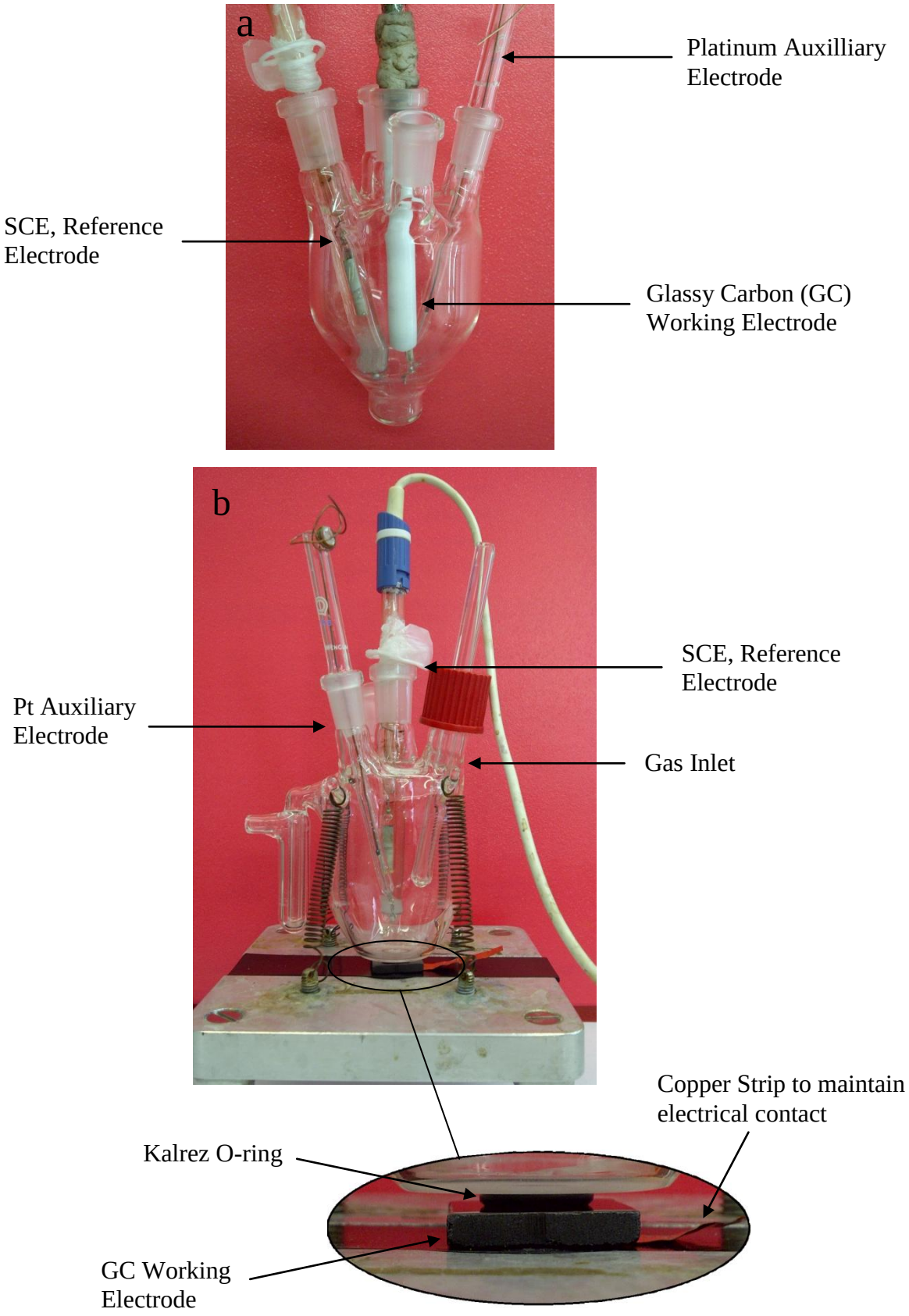


Figure 2.3. Cell setup for: a) a standard pear shaped 3 electrode cell; b) a pear shaped 3 electrode cell with a hole in the base.

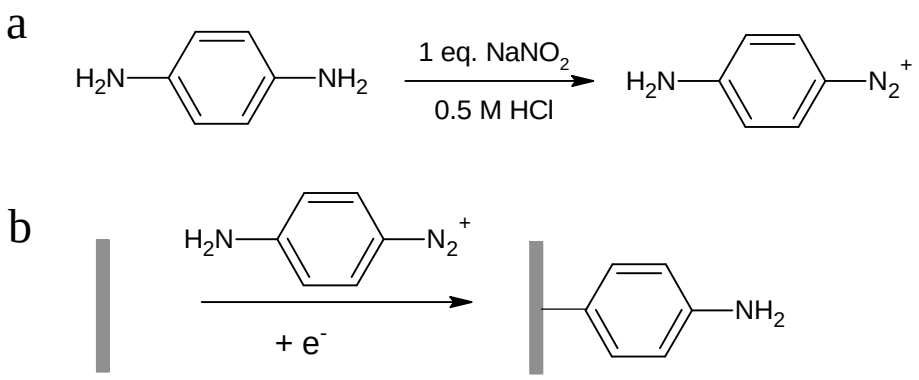
2.4. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was carried out using a JEOL JSM-7000F field emission scanning electron microscope. An accelerating voltage of 15 kV was used for all of the imaging done in this work.

2.5. General Surface Modification Procedures

2.5.1. Modification of Surfaces with Aminophenyl film

The GC electrode was modified by reduction of in situ generated aminobenzene monodiazonium cations using the method developed by Belanger and co-workers.[4] *p*-Phenylenediamine was reacted with an equal amount of sodium nitrite in 0.5 M HCl for 3 minutes (degassed with N_2) in an electrochemical cell to generate aminophenyl diazonium cation in situ (Scheme 2.1a). Immediately after 3 minutes, the GC working electrode was inserted into the cell, and grafting was performed electrochemically either using a chronocoulometry method or a cyclic voltammetry method. The electrochemically reduced aminophenyl diazonium cations were then grafted onto the GC surface (Scheme 2.1b). Different grafting conditions used to obtain different film thickness are summarised in Table 2.1. After modification, the GC was sonicated in MQ water for 5 minutes.



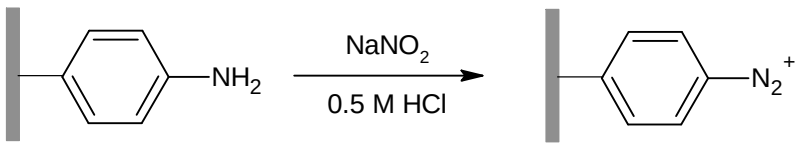
Scheme 2.1. a) In situ generation of diazonium cations; b) grafting of aminophenyl films on GC surface.

Table 2.1. Summary of different grafting condition used in this work.

Grafting Conditions				
	Concentration of reactant used	**Electrochemical method**	**Applied Potential**	**Modification time**
I	1 mM	Chronocoulometry	-0.1 V	120 s
II	10 mM	Chronocoulometry	-0.05 V	Cut off when charge reached -0.5 mC (~ 200 s)
III	0.2 mM	Chronocoulometry	-0.6 V	120 s
IV	1 mM	Cyclic Voltammetry	0.5 to -1.0 V	30 s
V	1 mM	Chronocoulometry	-0.6 V	120 s
VI	1 mM	Cyclic Voltammetry	0.5 to -1.0 V	120 s
VII	10 mM	Chronocoulometry	-0.6 V	120 s

2.5.2. Sticky Surface Formation via Aryldiazonium Salts (1)

In method (1), the sticky surface was obtained using modification of a method described by Viel and co-workers.[5] Aminophenyl modified GC electrode (Section 2.5.1) was immersed in solution of sodium nitrite (10 mM, in 0.5 M HCl) for 5 minutes to produce the diazonium terminated surface (Scheme 2.2). Sticky surface was washed with MQ water and dried under a stream of nitrogen prior to use.



Scheme 2.2. The conversion of aminophenyl films to sticky surface by treatment with sodium nitrite in acidic media.

2.5.3. Gooding and Co-workers[6] Method for Sticky Surface Formation (2)

Method (2) followed the procedure described by Gooding and co-workers[6] 1 mM *p*-phenylenediamine was reacted with 1 mM $NaNO_2$ in 0.5 M HCl for 10 minutes at 0 ^oC (degassed with N_2) to give aminobenzene monodiazonium cations. Electrografting was carried out by scanning in a potential range between 0.6 and -1.0 V for two cycles at a scan rate of 100 mV s^{-1}. After modification, the GC was sonicated in MQ water for 5 minutes. The modified GC was then immersed in 5 mM $NaNO_2$/0.5 M HCl for 15 minutes to obtain the sticky surface. Sticky surface was washed with MQ water and dried under a stream of nitrogen prior to use.

2.6. Film Thickness Measurements by Atomic Force Spectroscopy (AFM)

Film thickness measurements were carried out by Dr. Paula Brooksby using a previously described instrumentation and procedures.[7] For these measurements, AP films were grafted to pyrolysed photoresist film (PPF) surfaces, prepared by Dr. Paula Brooksby as described previously.[7]

2.7. Linkfit Software

Voltammetric peak areas were determined by using Linkfit curve fitting software.[8] Third or fourth order polynomial baselines were fitted to the voltammogram to determine a baseline for the voltammograms. Lorentzian, Gaussian, or a mixture of Lorentzian-Gaussian peaks were subsequently fitted to the voltammetric peak via the Lovenberg-

Marquardt algorithm. The peak area was determined by integration. An example of how the curve is fitted to the voltammetric peak is shown in Figure 2.4.

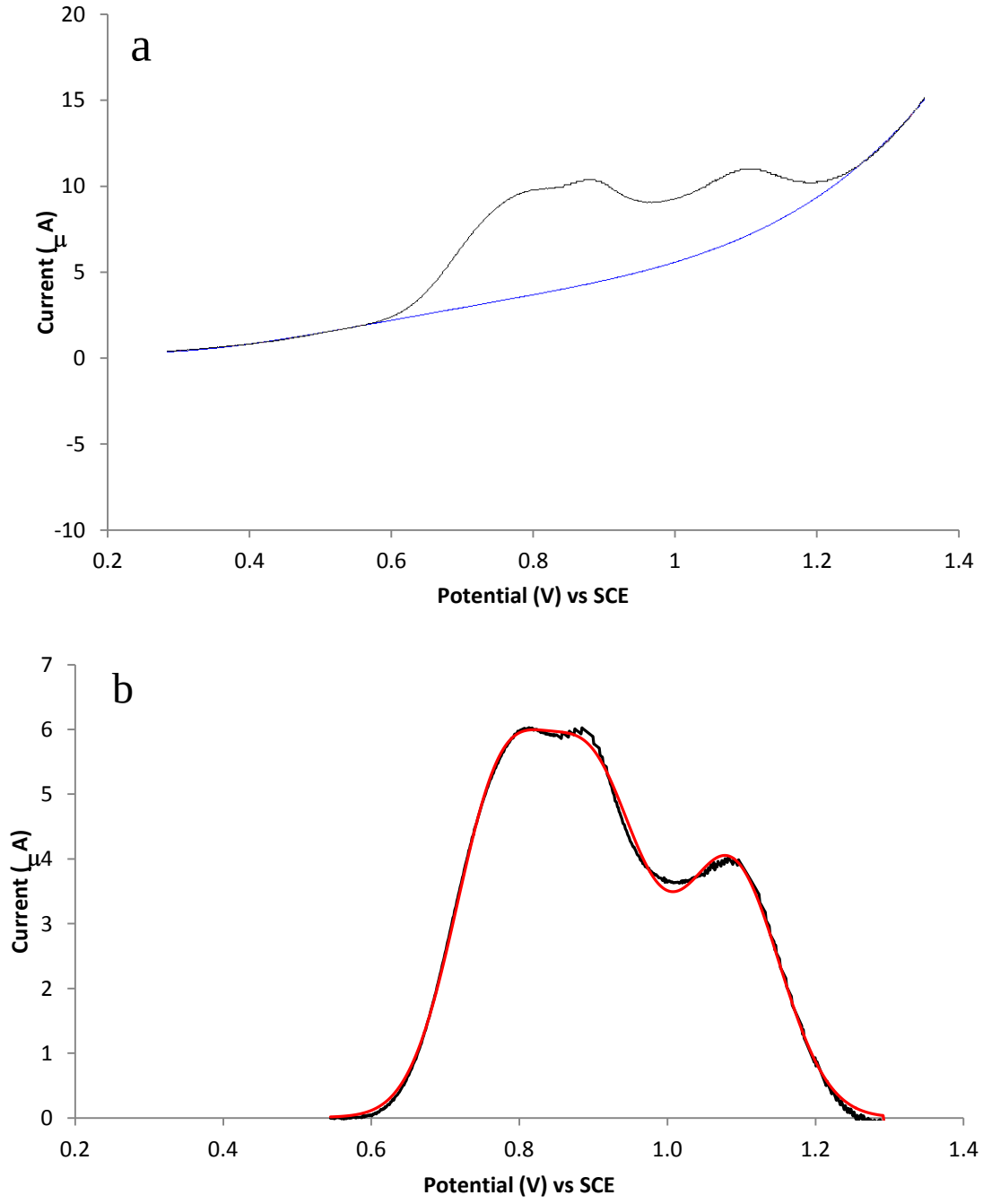


Figure 2.4. a) A fourth order polynomial baseline (blue line) was fitted to the voltammogram of the AP film oxidation peaks (black line). b) A plot showing how the voltammogram was fitted after the baseline correction was performed. The black line represents the actual voltammogram peaks and the red line represents the fitted curves.

2.8. References

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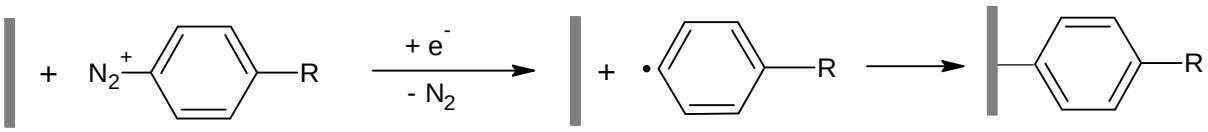
Chapter 3. Modification of Glassy Carbon by Electro-Reduction of the Aminobenzene Cation

3.1. Introduction

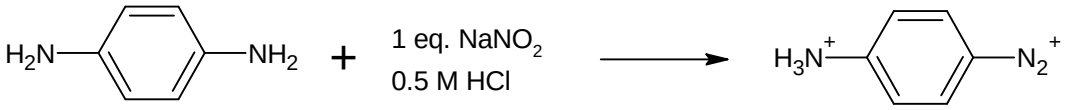
As described in Chapter 1, electrochemical modification of carbon surface using an aryldiazonium salt results in the formation of an aryl radical that reacts with the surface, leading to the formation of a C−C bond between the surface and the modifier layer[1] (scheme 3.1). It has been shown by a number of workers that multilayer formation is possible by electro-reduction of diazonium salts.[2-8] Formation of multilayers is proposed to proceed via the reaction of aryl radicals with other aryl groups that already bound to the surface.[3, 5, 8] Experimental conditions such as the concentration of modifier, electrolysis time and applied potential can be used to control film thickness.[7, 9]



Scheme 3.1. Reduction of aryldiazonium ion leading to covalent attachment of an aryl group on a surface.

One method to characterise films formed using aryldiazonium salts is to modify the surface with electro-active functionalities that can be detected electrochemically.[1, 3-5, 10-12] In this chapter, the grafting of aminobenzene diazonium ion to glassy carbon (GC) is investigated. The aminophenyl (AP) surface is electro-active, therefore the characterisation of the modified GC surfaces can be conducted electrochemically. The aim of this chapter is to gain a better understanding of AP film grafted onto GC via the in situ reduction of aryldiazonium salts. In situ formation of aminophenyl monodiazonium

cations can be achieved by addition of equal amount of $NaNO_2$ to *p*-phenylenediamine in acidic condition (Scheme 3.2).[13] Belanger and Lyskawa[13] have confirmed by gas chromatograpy-mass spectrometry that the formation of the monodiazonium cations from the *p*-phenylenediamine are preferred upon addition of one equivalent of $NaNO_2$ in the solution rather than the bisdiazonium cations. This is because the second amine group of the aminophenyl monodiazonium will be less reactive toward diazotation.



Scheme 3.2. In situ generated aminophenyl monodiazonium cations.

3.2. Experimental

Conditions used for the surface modification procedures are described in section 2.5.1. All experiments reported in this chapter were conducted using GC disk electrodes (area = 0.071 cm^2) as the working electrodes, Pt mesh as auxiliary electrode and SCE as reference electrode. Electrochemistry of amine modified GC surfaces was investigated in 0.1 M H_2SO_4. The ferricyanide redox probe solution was prepared using 5 mM of $K_3Fe(CN)_6$ in PBS (pH ~ 7). The ruthenium hexammine redox probe solution was prepared using 5 mM of $Ru(NH_3)_6Cl_3$ in 0.1 M HCl. Cyclic voltammograms (CVs) were recorded at a scan rate of 100 mV s^{-1}, unless specified otherwise.

3.3. Results and Discussion

3.3.1. Electrografting of AP films on GC

Electrochemically induced modification of GC with AP film was conducted in situ by recording cyclic voltammetric scans in the presence of 10 mM *p*-phenylenediamine and 10 mM $NaNO_2$ in 0.5 M HCl (as explained above). Figure 3.1 shows the irreversible reduction peak at $E_{p,c} \approx +0.1$ V (black line) assigned to the reduction of aminobenzene diazonium cation in protic conditions. The disappearance of the reduction peak on the second scan (red line) is consistent with the formation of grafted layers, which restrict further electrochemical reduction of the aminobenzene diazonium cation by inhibiting electron transfer from the electrode surface to aminobenzene diazonium cation in solution. This reduction peak of aminobenzene diazonium cation has been previously reported by Belanger and Lyskawa.[13] It is worth noting however that the peak at 0.1 V is relatively small and is superimposed on a broad 'background' redox process, which extends to at least -0.7 V. It is possible that all the reduction current seen over this potential range in the first scan is due to reduction of diazonium ions. This might arise due to grafting during the scans: as the scan progresses, the surface film becomes thicker and increasingly hinders electron transfer to diazonium ion in solution, effectively making the 'grafting potential' more negative throughout the scan.

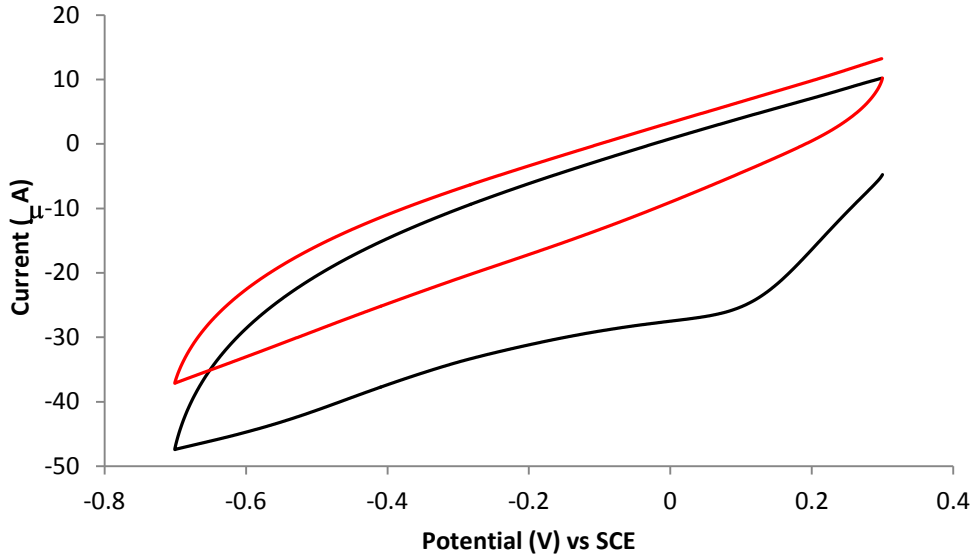


Figure 3.1. Electrochemical reduction of aminobenzene diazonium cation in a solution of 10 mM *p*-phenylenediamine + 10 mM $NaNO_2$ in 0.5 M HCl solution. The black line represents the 1st scan and the red line represents the 2nd scan.

3.3.2. Characterisation of AP Films

GC electrodes electrochemically modified with AP functionalities were investigated by assessing their blocking properties and the amine oxidation signal of the modified surfaces as discussed in the following sections.

3.3.2.1. Effect of AP Modification on Redox Probe Voltammetry

The presence of a film on the GC electrode surface was studied using redox probe voltammetry. Ferricyanide ($K_3Fe(CN)_6$, 5 mM, in 0.05 M PBS, pH 7, solution was usually used as the redox probe, although for some experiments a ruthenium hexammine solution ($Ru(NH_3)_6Cl_3$, 5 mM, in 0.1 M HCl) was used. Redox probe voltammetry is a useful technique for confirming the presence of a blocking film on the electrode surface.[14, 15] The presence of a film will decrease the rate of electron transfer between the electrode and the redox probe, which will give rise to an increase in the peak separation

(ΔE_p) and a decrease in the peak current. The more blocking the surface film is towards electron transfer to the redox probes, the larger the ΔE_p value.[10, 14-16] Therefore, the thick film is expected to lead to larger ΔE_p. As shown in Figure 3.2, the thicker film gives a larger ΔE_p value for CVs of $Fe(CN)_6^{3-}$ (Figure 3.2a) and $Ru(NH_3)_6^{3+}$ (Figure 3.2b) compared to the thinner film, which indicates that the thicker film is more blocking than the thinner film, as expected. At the electrodes modified with thick films, no peaks are observed in the potential window indicating very slow rates of electron transfer. The thinner and thicker film was prepared using grafting conditions II and VII, respectively, as described in Chapter 2, section 2.5.1.

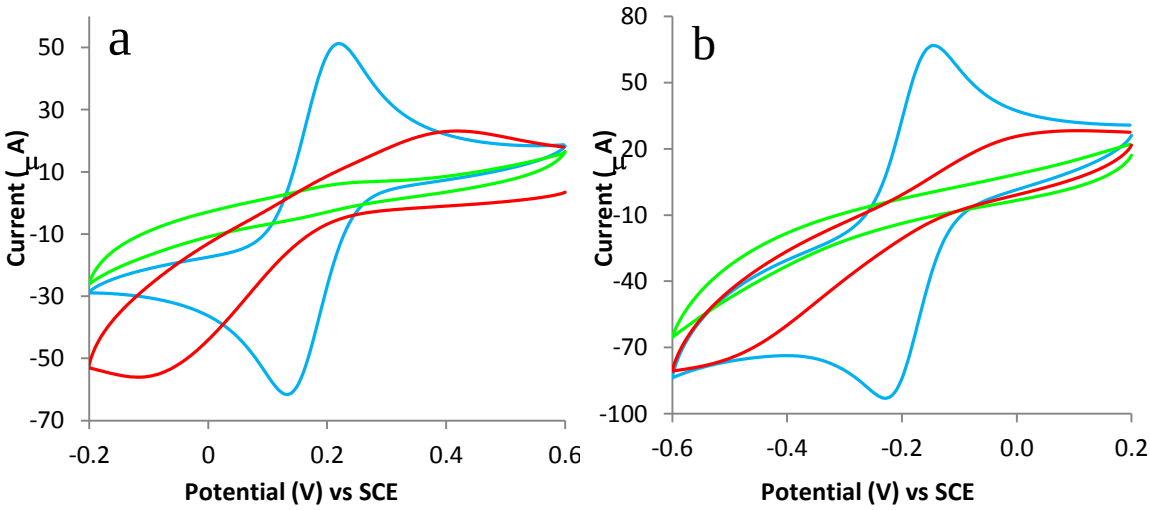


Figure 3.2. CVs showing the blocking effect of AP film at GC in: a) 5 mM $K_3Fe(CN)_6$ in PBS, and b) 5 mM $Ru(NH_3)_6Cl_3$ in 0.1 M HCl. The blue line is a scan taken before grafting, the red line is a scan taken after grafting of a thin film, and the green line is a scan taken after grafting of a thick film.

3.3.2.2. Electrochemistry of AP Modified GC

An AP film on a GC surface can be directly detected in protic conditions using cyclic voltammetry. In protic conditions AP films exhibit a broad irreversible oxidation manifold as seen in the voltammogram in Figure 3.3 (black line), where on the second scan, the oxidation peaks have completely disappeared (red line). However, sometimes

these oxidation peaks do not completely disappear on the second scan (Figure 3.4, red line), suggesting that not all of the amine functionalities have been oxidised in the first scan. At least three oxidation peaks can be seen in the CVs (Figure 3.4, labelled I, II and III). The origin of these multiple peaks is not known. It is possible that they arise from AP groups in different film environments, but it is also possible that they are products of reactions of AP groups during the preparation of the film. These three peaks are seen more clearly for films for which the concentration of the diazonium cation solutions used in the grafting process are more dilute.

In protic solution, oxidation of aniline leads to polymerisation with the formation of polyaniline.[17, 18] When AP groups are anchored in a multilayer film, extensive polymerisation through the amine groups is not possible but some formation of oligomers might account for the small redox couple with $E_{½} \approx 0.55$ V, seen on the second scan (Figure 3.3 and 3.4). The major product(s) from the oxidation of the film are not known, and the number of electrons involved, per amine group, is also not known. Because the nature of the redox reaction(s) is unknown, the estimation of the surface concentration of electro-active AP groups is not possible. However, the AP film before and after the oxidation process was studied by redox probe voltammetry as discussed in the following section.

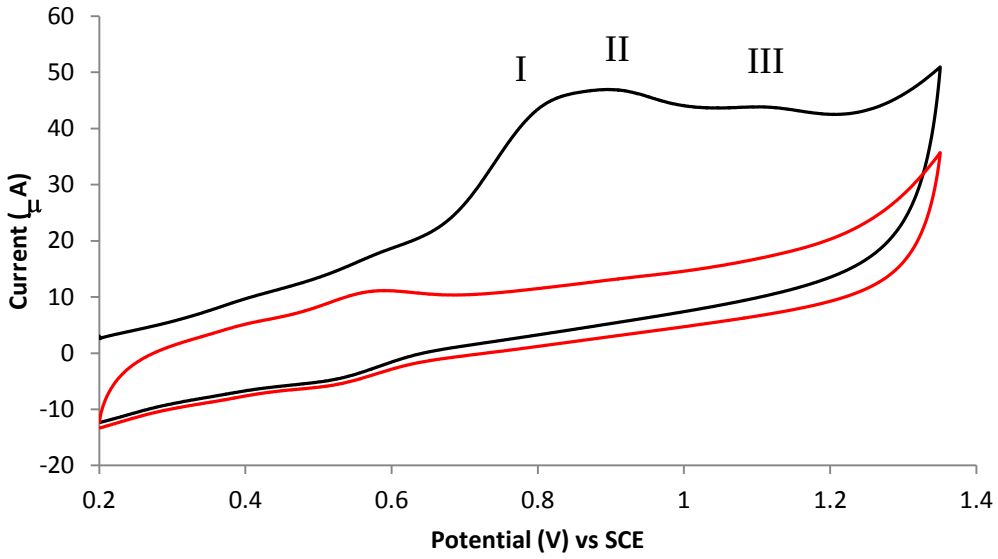


Figure 3.3. CV of AP modified GC (grafting condition VII) in 0.1 M H_2SO_4 showing the irreversible redox process of amine oxidation. The black line represents the 1st scan and the red line represents the 2nd scan.

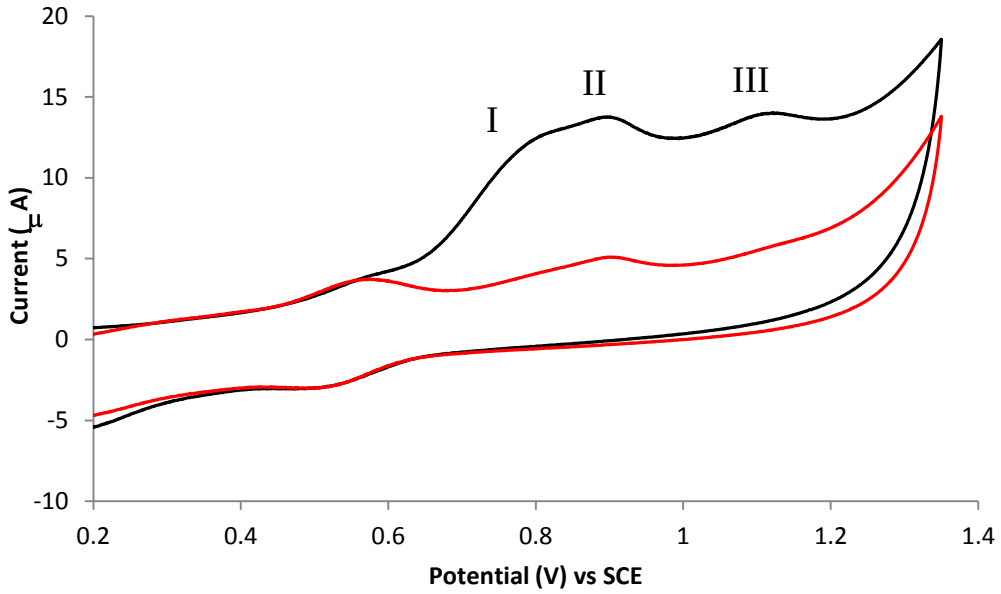


Figure 3.4. CV of AP modified GC (grafting condition V) in 0.1 M H_2SO_4 showing the irreversible redox process of amine oxidation at $E_{p,a} \approx 0.84$ V. The black line represents the 1st scan and the red line represents the 2nd scan.

3.3.2.3. Effect of AP Film (Before and After Oxidation) on Redox Probe Voltammetry

Comparison of the cyclic voltammetric response of solution redox probes at oxidised and non-oxidised AP film electrodes was used to investigate the AP film. Figure 3.5 shows CVs recorded at bare GC (blue line), AP modified GC (red line), and oxidised AP modified GC (green line), in the presence of (a) $Fe(CN)_6^{3-}$ and (b) $Ru(NH_3)_6^{3+}$. The CV recorded at the bare GC electrode shows the quasi-reversible $Fe(CN)_6^{3-}$ redox couple with a peak separation ΔE_p = 86 mV. Upon modification with an AP film, the peak separation increases to ΔE_p = 195 mV and the peak current has decreased, indicating a decrease in the rate of electron transfer to and from $Fe(CN)_6^{3-/4-}$. As discussed above, this response is evidence for the presence of an insulating film at the electrode surface. When this film was electrochemically oxidised, the $Fe(CN)_6^{3-/4-}$ redox couple is seen to disappear almost entirely, suggesting that the oxidised AP film inhibits electron transfer more strongly than the non-oxidised AP film.

On the other hand, when an opposite charged (positive) redox probe is used, such as $Ru(NH_3)_6^{3+}$, an opposite effect was observed. As shown on Figure 3.5b, the CV recorded at the bare GC electrode shows the quasi-reversible $Ru(NH_3)_6^{3+}$ redox couple with a peak separation ΔE_p = 85 mV, which upon modification with AP film, disappears almost entirely. When this film was electrochemically oxidised, an increase in the rate of electron transfer to and from $Ru(NH_3)_6^{3+/2+}$ is seen with ΔE_p = 232 mV for the cyclic voltammogram.

The pK_a of the AP modified film is not known. Aniline has a pK_a of 4.6. The pK_a of a chemical species in solution can be different from the one on a solid support.[19, 20]

Previous studies have shown that the electrochemistry of $Fe(CN)_6^{3-}$ is severely hindered at a neutral or anionic surface film.[15, 21] Hence, based on the scans shown in Figure 3.5a, it appears that the AP film before oxidation has a nett positive charge at pH 7. The positively charged AP film promotes close approach of $Fe(CN)_6^{3-}$ to the surface, facilitating fast electron transfer. After oxidation, $Fe(CN)_6^{3-}$ is clearly no longer attracted to the surface, suggesting that the AP film has either neutral or negative charge upon electrochemical oxidation in protic condition. In contrast, the positively charged AP film in acidic condition will repel $Ru(NH_3)_6^{3+}$ from the surface, decreasing its electron transfer rate (Figure 3.5b). After oxidation of the film, $Ru(NH_3)_6^{3+}$ can approach more closely to the surface, consistent with a neutral or negatively charged surface.

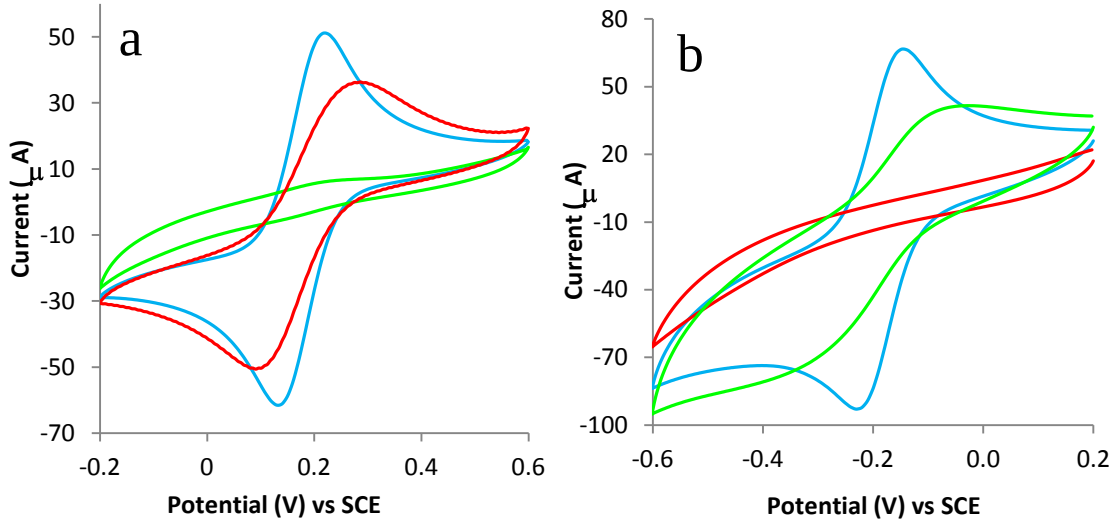


Figure 3.5. CVs showing the opposite blocking effect of GC in: a) 5 mM $K_3Fe(CN)_6$ in PBS (pH 7), and b) 5 mM $Ru(NH_3)_6Cl_3$ in 0.1 M HCl. The blue line is a scan taken before grafting, the red line is a scan taken before oxidising the amine, and the green line is a scan taken after the amine had been oxidised.

3.3.3. Varying the Surface Concentrations of Amine Film

Factors that can influence the surface concentration of films formed by electrochemical methods include the concentration of modifier, the applied potential and the electrolysis modification time.[3, 5] In this work preparation of AP films with different surface concentrations were investigated as described in the following sections.

3.3.3.1. Varying the Concentration

The diazonium ions used in the grafting process are produced in situ by reaction of *p*-phenylenediamine and one equivalent of $NaNO_2$ in 0.5 M HCl. Different concentrations of *p*-phenylenediamine were used to obtain different surface concentrations of the AP film; as the modifier concentration increases so too will the surface concentration. It is commonly observed however that films electrografted from diazonium salt solutions have a limiting thickness. The limiting thickness is reached when the rate of electron transfer across the film, to the diazonium cations in solution, becomes negligible.[5]

To assess the relative surface concentration of films formed under different conditions, the areas under the amine oxidation peaks can be compared. (The area under the peak corresponds to the charge required to oxidise AP groups.) This comparison assumes that all AP groups in the films are electroactive, or that the same proportions of groups are electroactive in films of different surface concentrations. Work with other diazonium grafted films has shown that typically not all groups are electroactive, particularly in thick films,[22, 23] and hence the area of the amine oxidation peak can give a qualitative indication of surface concentration only.

Figure 3.6 shows CVs of GC modified with AP films using different concentration of *p*-phenylenediamine (0.2 mM, 1 mM and 10 mM). Grafting was carried out by the

chronocoulometry method setting the potential at -0.6 V for 120 seconds. As the concentration of reactants increases, so does the amine oxidation peak; this indicates that as the concentration of diazonium cation increase so does the film surface concentrations. These CVs show that there is only a small increase in area of the amine oxidation peak when the concentration is increased from 0.2 mM to 1 mM, and a large increase when 10 mM solution was used.

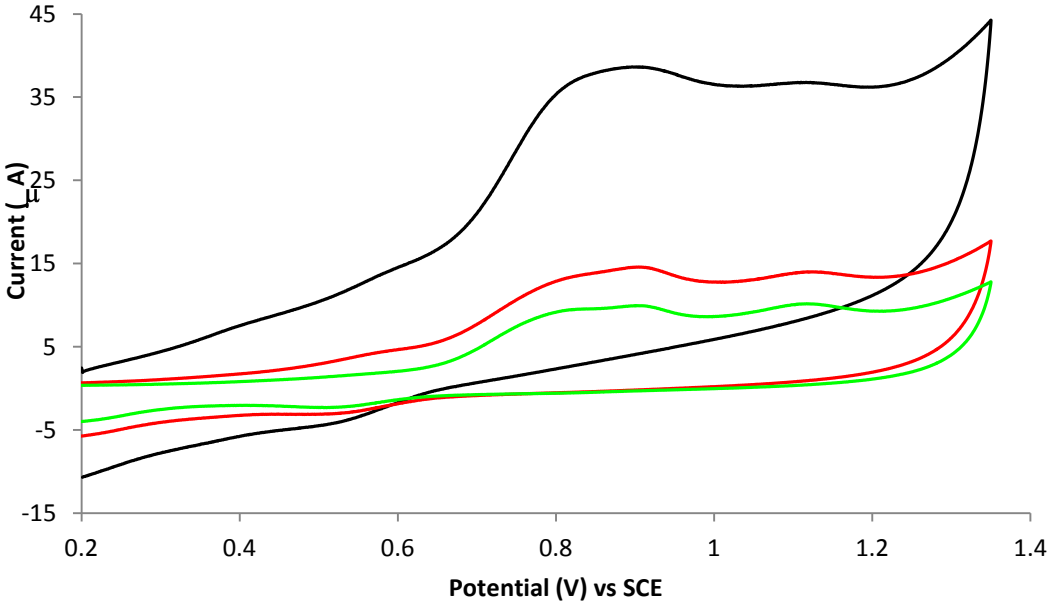


Figure 3.6. CVs of AP modified GC in 0.1 M H_2SO_4 showing the amine oxidation for different film thickness. The GC grafted with 0.2 mM, 1 mM and 10 mM diazonium solution was represented by the green line, red line and black line respectively.

3.3.3.2. Varying the Applied Potential

Besides the concentration of diazonium ions, the applied potential can also be used to vary the surface concentration of the grafted film. Grafting was carried out by the chronocoulometry method by applying the potential for 120 seconds in a solution of 1 mM *p*-phenylenediamine and 1 mM $NaNO_2$ in 0.5 M HCl. Figure 3.7 shows CVs of GC modified with AP films using different applied potentials (-0.1 V and -0.6 V). At the more negative applied potential, the amine oxidation peak is significantly larger than the

one at the less negative potential. As the applied potential becomes increasingly negative, the rate of electron transfer to diazonium ion in solution increases exponentially.[24] In addition, if the films are reaching their limiting thickness, more negative potential gives a thicker film (higher surface concentration) because electron transfer across the film to diazonium ion in solution can occur across a thicker film when the potential is more negative.[25]

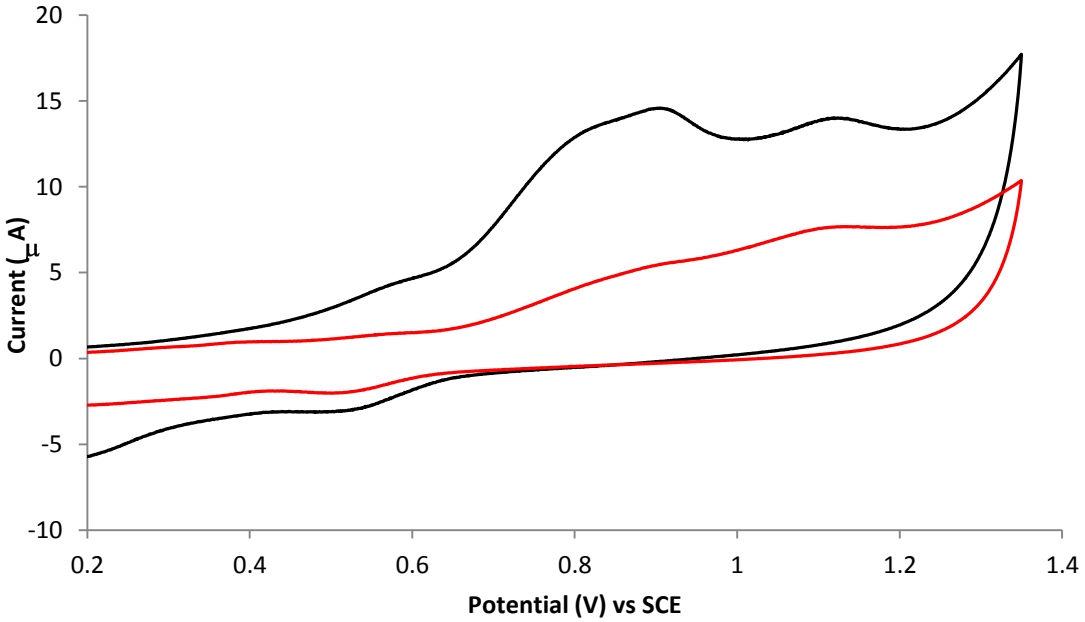


Figure 3.7. CVs of AP modified GC in 0.1 M H_2SO_4 showing the amine oxidation for different film thickness by varying the applied potential during grafting process. The red line represents a -0.1 V grafting potential and the black line represents a -0.6 V grafting potential. Grafting was carried out for the duration of 120 s using chronocoulometry method.

3.3.3.3. Varying the Scan Cycles

Cyclic voltammetric scans (from +0.5 to -1.0 V, scan rate = 100 mV s^{-1}) were used to modify the GC surface in the presence of 1 mM AP diazonium ion. The surface concentration was controlled by the number of scan cycles performed. Figure 3.8 shows CVs of GC modified with AP films using different numbers of scan cycles (1 vs. 4). As

the number of scan cycles increase so does the oxidation peak; this indicates that as the number of scan cycles increase so does the film surface concentration.

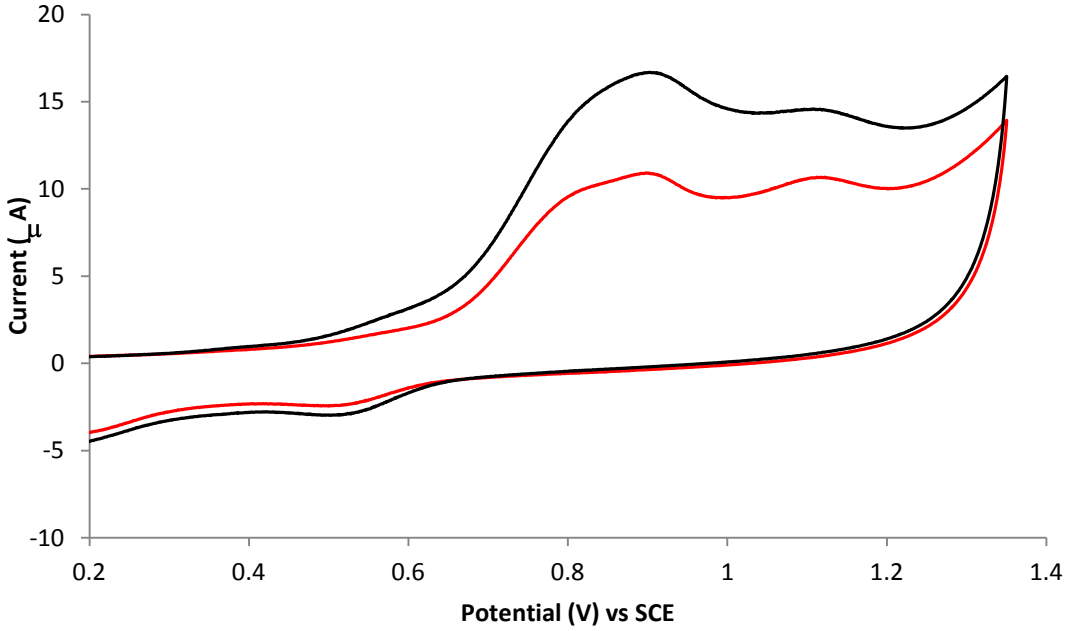


Figure 3.8. CVs of AP modified GC in 0.1 M H_2SO_4 showing the amine oxidation for an AP film grafted with 1 cycle scan (red line) and 4 cycles scan (black line). Grafting was carried out by scanning between 0.5 and -1.0 V at a scan rate of 100 mV s^{-1}.

3.3.4. Summary of Effect of Film Preparation Conditions and Reproducibility of AP Modified GC

Table 3.1 summarises the data obtained for the various film preparation conditions. The area of the voltammetric peaks from the amine oxidation was calculated using Linkfit software[26] and the standard error was calculated. Because the reduction of the AP film is not fully understood, the estimation of the surface concentration of electro-active AP groups is not possible. Therefore, the charges associated with each peak (calculated from the peak areas of the amine oxidation for each film) are compared.

For two films, the thickness was also measured by depth-profiling using the atomic force microscope (AFM). The AFM measurements were carried out by Dr. Paula Brooksby. To measure the thickness of a very thin film, a very smooth substrate is required, therefore

films were grafted using conditions V and VII to pyrolyzed photoresist film (PPF). PPF is a ultra flat GC-like material, synthesised in house by Dr. Paula Brooksby. The typical root mean square (rms) surface roughness of PPF is < 0.5 nm. Table 3.1 shows that AP film grafted using condition V has a thickness of 1.5 ± 0.2 nm and grafting with condition VII gives 5.1 ± 0.2 nm. These values for the film thickness have been adjusted for the scratching depth of bare PPF (0.3 nm). A monolayer of AP groups (upright on the surface) has a thickness of about 0.7 nm.[3] Hence, these films are multilayered with at least 2 and 7.5 monolayer equivalents, for grafting condition V and VII respectively. These results show that the charge associated with the amine oxidation is related to the film thickness: lower surface charge is associated with a thinner film and higher surface charge with a thicker film. Comparing the relative thickness and relative charges of these films, condition VII gives a film that is approximately three times thicker than condition V, but the charges associated with these films differ by only a factor of two. This confirms the findings of other workers[4, 22] that at least for relatively thick film, not all of the functionalised groups of the film are electroactive.

Consideration of the charge data in Table 3.1 highlights that concentration of diazonium ion is important in determining the amount of film formed (compare grafting conditions III, V and VII), as is the lower potential limit (compare grafting conditions I and V). The reproducibility of film formation can also be assessed by these data. The nature of the radical reaction means diazonium film preparation can be quite variable, because it can be difficult to control a radical reaction precisely. Therefore, for the reproducibility test, three independent experiments were carried out for each film preparation method. Standard errors (Table 3.1) were calculated from the measured charges of the three replicate films of each type. The standard errors show that the surfaces modified using this method are only moderately reproducible. Note that these errors do not account for

the uncertainties surrounding how the baseline is positioned. The second scan cannot serve as an accurate baseline because during the recording of cyclic voltammograms, the film structure is constantly changing, hence the baseline for the peak is changing as well. The same method was used to determine the baseline for all films (see section 2.7) so that relative peak sizes could be compared.

Table 3.1. Summary of the charge associated with amine oxidation in the films after each of the different methods of preparation, and the associated calculated uncertainties.

Grafting condition	Film thickness (± 0.2 nm)	Charge associated with amine oxidation (μC)					
		1st expt	2nd expt	3rd expt	Average	St. dev	St. error
I) 1 mM solution, potential set at -0.1 V for 120 s	-	9.8	10.1	11.7	10.6	1.0	9.7 %
II) 10 mM solution, *E* = -0.05 V, stopped when charge reached -0.5 mC	-	12.4	14.6	14.3	13.8	1.2	8.7 %
III) 0.2 mM solution, potential set at -0.6 V for 120 s	-	19.4	16.3	18.8	18.2	1.6	9.1 %
IV) 1 mM solution, scanned 1x from 0.5 to -1.0 V at 100 mV s^{-1}	-	23.8	25.2	24.6	24.5	0.7	3.0 %
V) 1 mM solution, potential set at -0.6 V for 120 s	1.5	24.8	28.6	27.4	26.9	1.9	7.1 %
VI) 1 mM solution, scanned 4x from 0.5 to -1.0 V at 100 mV s^{-1}	-	34.4	32.2	32.0	32.8	1.3	4.0 %
VII) 10 mM solution, potential set at -0.6 V for 120 s	5.1	57.5	54.9	54.8	55.7	1.5	2.7 %

3.4. Conclusions

Modification of GC with AP films was performed by reduction of in situ generated aminobenzyl monodiazonium cations in solution. Electrochemistry was used to investigate the AP modified GC surface, as AP groups are electro-active species with irreversible redox systems. The product(s) of electro-oxidation of the AP film is not known, therefore, the estimation of the surface concentration of electro-active AP groups is not possible.

The oxidation of the AP groups is irreversible, making repeat measurements impossible and allowing for only "one shot" at determination of the charges associated with redox processes of the film. Therefore, it is important to have a reproducible result. From the three separate experiments carried out on each thicknesses of each modified surface, it was shown that the surface modifications are moderately reproducible allowing comparison between each film preparation method to be made.

The redox probes $K_3Fe(CN)_6$ and $Ru(NH_3)_6Cl_3$ were used to verify surface modification of GC with AP film. Moreover, the AP modified surface before and after electrochemical oxidation in protic condition was also investigated using those redox probes. Studies showed that after electro-oxidation, the positively charged AP groups have been converted to a group that is either neutral or negatively charged. The products after the electro-oxidation will not be investigated in this thesis.

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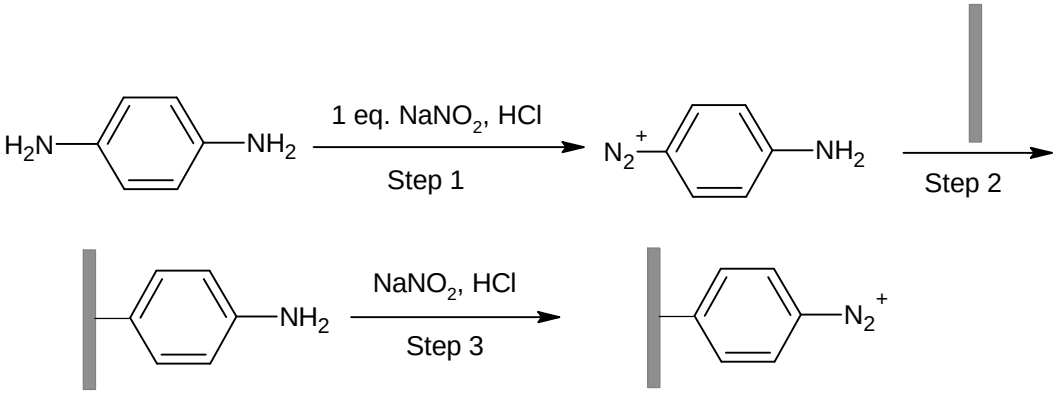
Chapter 4. Formation and Reactions of Sticky Surface with Nanoparticles and Aniline

4.1. Introduction

As mentioned in Chapter 1, "sticky surface" is a term used to describe a surface containing a terminal diazonium functionality. The diazonium functionality is usually obtained from a two diazotisation-step protocol based on *p*-phenylenediamine. The first diazotisation step is followed by reduction of the diazonium cation and grafting to give an aminophenyl (AP) layer (Scheme 4.1, step 2). This layer then undergoes second diazotisation by reaction with $NaNO_2$ and HCl to form the desired sticky surface (step 3).



Scheme 4.1. Reaction steps to form diazonium ions terminated surface (sticky surface).

As outlined in Chapter 1, sticky surface has been used to covalently graft to nanoparticles,[1-4] carbon nanotubes (CNTs),[4-8] nitrogenated organic compounds,[4, 9] enzyme[10, 11] and DNA or protein.[12, 13] Spontaneous reaction of sticky surface with many substrates is expected, since diazonium ions are very reactive and easily reduced. Moreover, electrochemical or photochemical activation can be employed to initiate a reaction with a less reactive substrate. Covalent immobilisation of gold nanoparticles

(Au-nps) onto a surface via diazonium chemistry has been reported.[1, 2] However, the attachment of these Au-nps was carried out by using either electrochemical or UV initiation to reduce the diazonium functionalities to the aryl radicals rather than spontaneous reduction by the Au-nps to form covalent bonds. Au-nps are not known to be good reducing agents or good nucleophiles, therefore a spontaneous reaction between Au-nps and the sticky surface might not be expected to proceed. On the other hand, spontaneous grafting of diazonium salts has been observed on gold surfaces.[14, 15]

In this chapter, the formation of diazonium cation terminated surface on GC is investigated. The diazonium cations formed are electro-active, therefore the characterisation of the sticky surface can be conducted electrochemically. The aim of this chapter is to gain a better understanding of sticky surface formation on GC surfaces and to further investigate the scope of reaction of sticky surfaces. First, electrochemical and spontaneous reactions of Au-nps with sticky surfaces are explored. In the second part of the work, the well known reaction of aniline with diazonium ion to form an azo dye was utilised. This reaction was expected to occur spontaneously at the sticky surface, to give an AP functionality that can be detected electrochemically.

4.2. Experimental

Sticky surfaces were prepared according to the procedures described in section 2.5.2. Electrochemically induced reaction of sticky surface with citrate-capped Au-np was undertaken by recording two consecutive cyclic voltammetric scans from +0.6 to -0.8 V vs. SCE in citrate-capped Au-np solution at a scan rate of 100 mV s^{-1}.[2] Spontaneous reaction of sticky surfaces with citrate-capped Au-np was carried out by immersion of the sticky surface in citrate-capped Au-np solution for 1 hour. The citrate-capped Au-np solution was prepared as described in section 2.2.2.1 and was used as prepared. After

modification with citrate-capped Au-nps, the surfaces were subjected to five minutes of sonication in Milli-Q (MQ) water and dried under a stream of nitrogen, unless specified otherwise. Spontaneous reaction of sticky surface with aniline was carried out by immersion of the sticky surface in aniline/ACN solution. After modification, the surfaces were subjected to five minutes of sonication in acetonitrile and dried under a stream of nitrogen.

All electrochemical experiments at glassy carbon (GC) were conducted using GC disk electrodes (area = 0.071 cm^2) as the working electrode, Pt mesh as auxiliary electrode and SCE as reference electrode. The experiments carried out for SEM imaging were conducted on GC plate electrodes using a 010 O-ring (Kalrez) to define the working area of the electrode (0.289 cm^2). The electrochemistry of GC modified with sticky surface was investigated in 0.1 M H_2SO_4. The electrochemistry of Au-np and aniline were also studied in 0.1 M H_2SO_4. All cyclic voltammograms (CVs) were recorded at a scan rate of 100 mV s^{-1}, unless specified otherwise.

4.3. Results and Discussion

4.3.1. Sticky Surface Formation

4.3.1.1. Characterisation of Sticky Surface

A sticky surface has a diazonium functionality terminating film, which is an electro-active species that can be detected electrochemically. It is assumed that one-electron reduction of the phenyldiazonium cation groups in the film leads to elimination of N_2, but the nature of follow-up reactions is unknown. Photodecomposition of phenyldiazonium groups has been suggested to lead to phenol groups.[12, 16, 17]

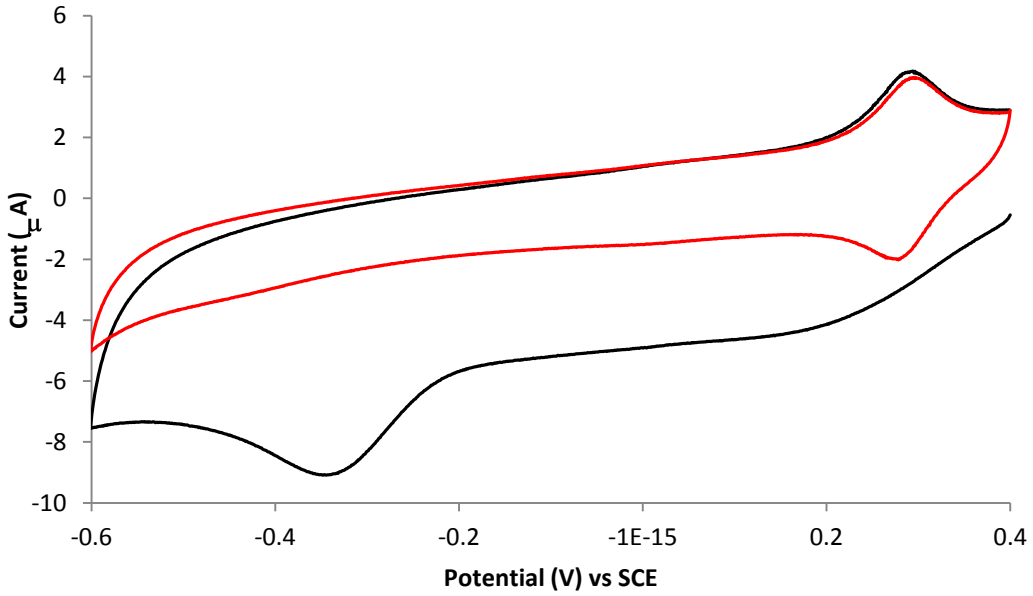


Figure 4.1. CV of sticky surface modified GC with grafting condition V in 0.1 M H_2SO_4. The black line represents the 1st scan and the red line represents the 2nd scan.

Figure 4.1 shows consecutive CVs obtained in 0.1 M H_2SO_4 of diazonium terminated films prepared from grafting condition V of the AP films on GC surface. Grafting condition V was performed by using the chronocoulometry method in a solution of 1 mM aminobenzenediazonium cation by holding a potential of -0.6 V for 120 s, followed by immersion in $NaNO_2$ for 5 minutes to obtain the sticky surface. Scanning in the negative direction from an initial potential of 0.4 V shows, in the first scan (black line), two reduction processes, a broad process starting at ~0.4 V and a well-defined peak with $E_{p,c}$ ≈ -0.35 V. Both of these peaks are not seen in the second scan (red line) indicating that they are chemically irreversible processes. However, the disappearance of these peaks gives rise to a redox couple with $E_{½}$ ≈ 0.3 V, seen on the second scan (Figure 4.1, red line).

Bare GC and GC modified with AP film were scanned in 0.1 M H_2SO_4 (Figure 4.2), to provide the blanks for this work. As shown in Figure 4.2, there is no peak at $E_{p,c}$ ≈ 0.2 V or $E_{p,c}$ ≈ -0.35 V for either the bare GC (red line) or the AP film (blue line).

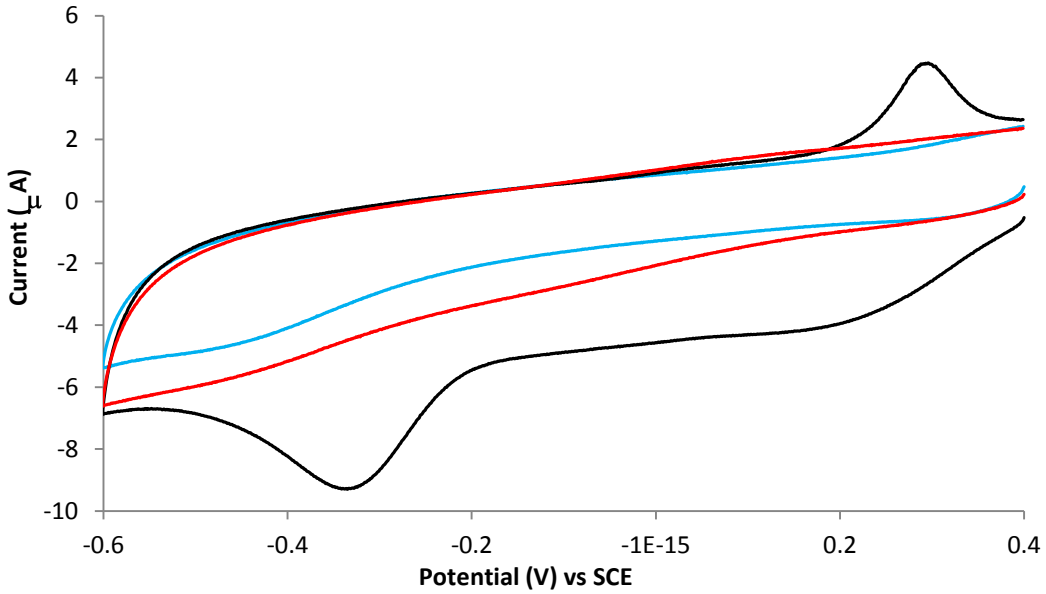


Figure 4.2. CV showing first scan of: sticky surface (black line), AP (blue line) modified with grafting condition V, and bare GC (red line) in 0.1 M H_2SO_4.

The CVs of the sticky surface were reproducible, however they are different to those reported previously. Figure 4.3 shows the CV obtained by Gooding and co-workers.[2] They state that diazonium terminated film is reduced in the range between 0 and -0.3 V, however they do not observe a well-defined peak at -0.35 V.

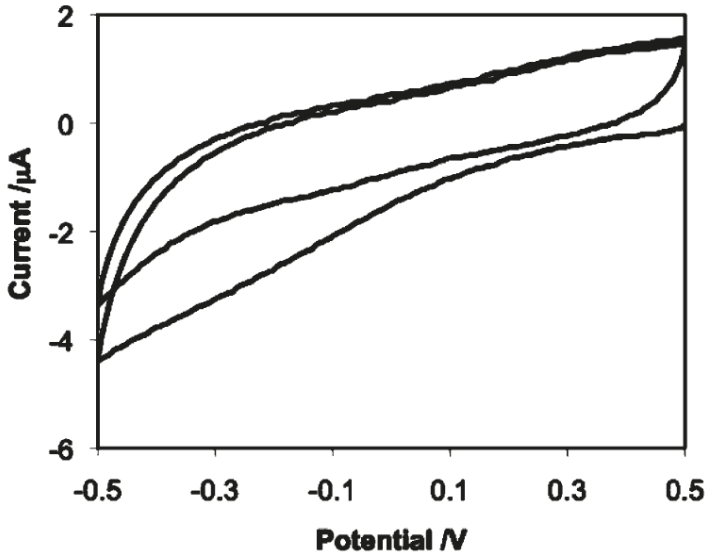
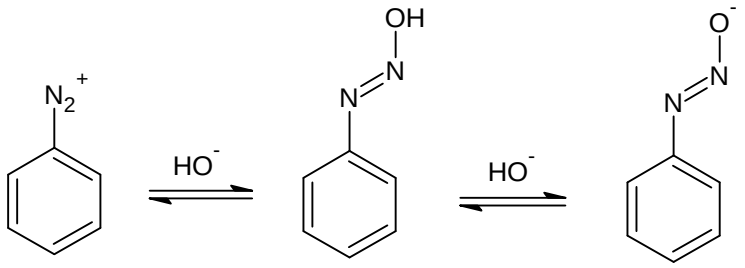


Figure 4.3. CVs of diazonium cation terminated surface in 0.5 M HCl and 1 mM $NaNO_2$ obtained by Gooding and co-workers.[2] Reproduced from reference 2.

To investigate the potential corresponding to diazonium reduction, the sticky surface was immersed in 0.01 M NaOH solution (pH ~ 11.6) for 1 hour (Figure 4.4). Under basic conditions, diazonium ions are converted to diazoates, which are unstable at pH > 10 (Scheme 4.2).[15] It is seen on Figure 4.4 that the broad process starting at ~0.4 V has disappeared, while the peak at $E_{p,c} \approx$ -0.35 V remains. This suggests that the broad process starting at ~0.4 V is due to the diazonium cations reduction and the peak at $E_{p,c} \approx$ -0.35 V is not due to the diazonium functionalities. The very broad nature of the peak assigned to diazonium reduction is presumed to be due to the presence of diazonium groups in different environments in the film. For example, diazonium groups may be at the surface and buried at different depths in the film. The permeability of the film to solvent and ions will vary throughout the film which is expected to affect the diazonium redox activity.



Scheme 4.2. Equilibrium of diazonium ions to its diazohydroxide and diazoates.

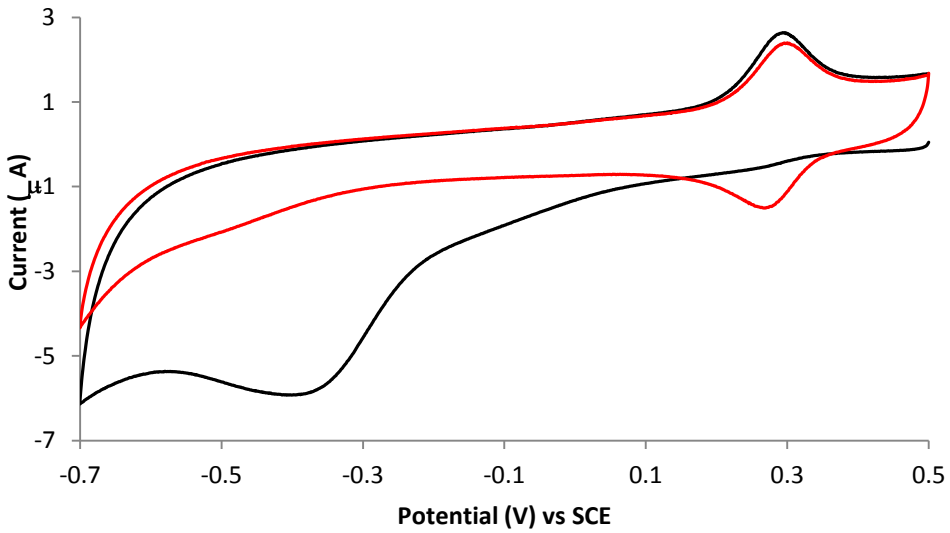
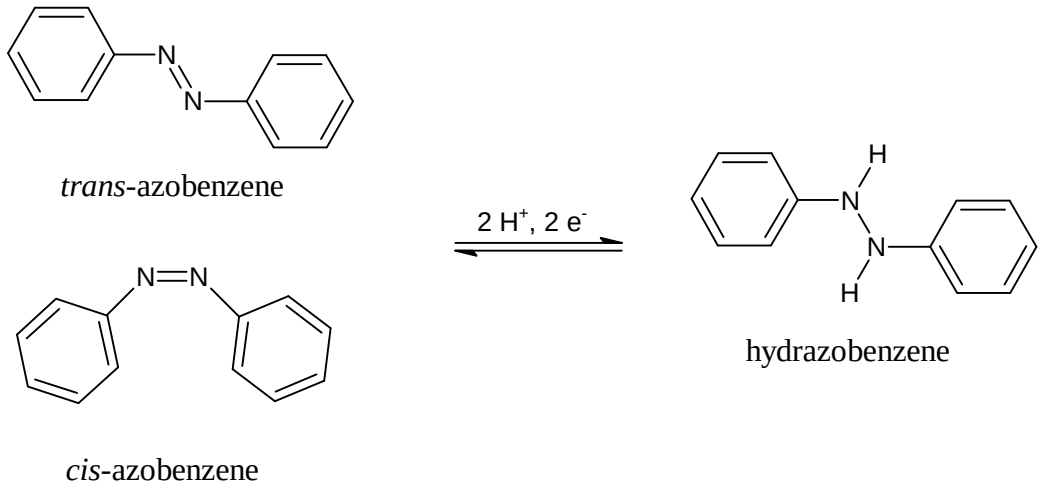


Figure 4.4. CV of sticky surface modified GC with grafting condition V in 0.1 M H_2SO_4 after subjected to 1 hour immersion in 10 mM NaOH (pH ~ 11.6). The black line represents the 1st scan and the red line represents the 2nd scan.

The nature of the species giving rise to the peak at $E_{p,c}$ ≈ -0.35 V is not known. This species could be formed during formation of the sticky surface or could arise from reduction (or decomposition) of the sticky surface (Figure 4.4). One possibility is that the species is an azobenzene, formed during preparation of the sticky surface. As sticky surface is formed, diazonium films are generated in the presence of AP groups and hence azo coupling could occur. While the film environment is confined, there is a high concentration of functionalities in the film and hence some reaction may be possible. Azobenzene can exist as *cis* and *trans* isomers, which can undergo a two-proton, two-electron reduction to give hydrazobenzene (Scheme 4.3). According to a paper by Compton and co-workers[18] the *trans* isomer is reduced at a more negative potential compared to the *cis* isomer, giving hydrazobenzene which upon reoxidation is converted to the more stable *cis* form to give a *cis*-azobenzene. The redox couple at $E_{½}$ ≈ 0.3 V (Figure 4.4) is similar to the redox couple of *cis*-azobenzene reported by Compton and co-workers.[18] Therefore, it is tentatively proposed that the cathodic peak at $E_{p,c}$ ≈ -0.35 V is due to the *trans*-azobenzene and the redox couple at $E_{½}$ ≈ 0.3 V is due to the *cis*-azobenzene.



trans-azobenzene

cis-azobenzene

hydrazobenzene

Scheme 4.3. Redox process of azobenzene isomers.

The redox couple at $E_{½} \approx 0.3$ V was further investigated to establish whether this species is formed from the redox process at $E_{p,c} \approx -0.35$ V or from the broad diazonium reduction starting at ~0.4 V. CVs scan were conducted by progressively stepping the negative potential limit for each scan to more negative potentials (Figure 4.5). These CVs clearly show that the redox couple with $E_{½} \approx 0.3$ V is only visible when the scan was performed to a more negative potential than the reduction peak at $E_{p,c} \approx -0.35$ V. This indicates that the redox couple with $E_{½} \approx 0.3$ V arises due to the reduction process at $E_{p,c} \approx -0.35$ V, which is in agreement with the proposed redox process of an azobenzene. However, the electrochemistry of the azo groups in films is complicated and various behaviours are reported in literature.[18-20] Therefore, a definite conclusion regarding the cathodic peak at $E_{p,c} \approx -0.35$ V and the redox couple at $E_{½} \approx 0.3$ V cannot be made and further investigations were not undertaken in this work.

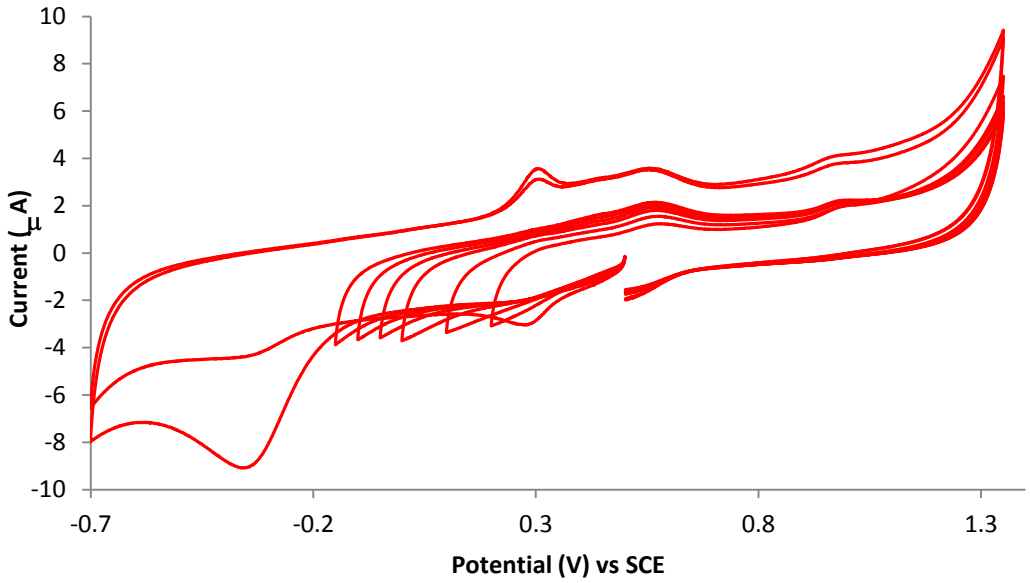


Figure 4.5. CV of sticky surface modified GC with grafting condition V in 0.1 M H_2SO_4.

4.3.1.2. Stability of Sticky Surface

Aryldiazonium salts are generally unstable compounds and GC is known to spontaneously reduce aryldiazonium cations in solution.[21] For a thin film sticky surface in

particular there is a possibility that the GC will spontaneously reduce the attached diazonium cations of the sticky surface giving aryl radicals. It is assumed that for a thicker film, electron transfer across the film is blocked, therefore, self reduction (by the surface) of the diazonium groups in the outer part of the film at least, is unlikely. Therefore, it is important to determine the stability of the sticky surface. A freshly prepared sticky surface was immersed in 0.1 M H_2SO_4 and sodium citrate (pH ~ 5.8) for 1 hour, in two separate experiments. Sodium citrate was included because it is a component of gold nanoparticle solutions which are used in later experiments with sticky surfaces (Section 4.3.2.3). Immediately after the 1 hour immersion, the modified electrode was washed with MQ water, dried with N_2 gas, and scanned for diazonium reduction (Figure 4.6).

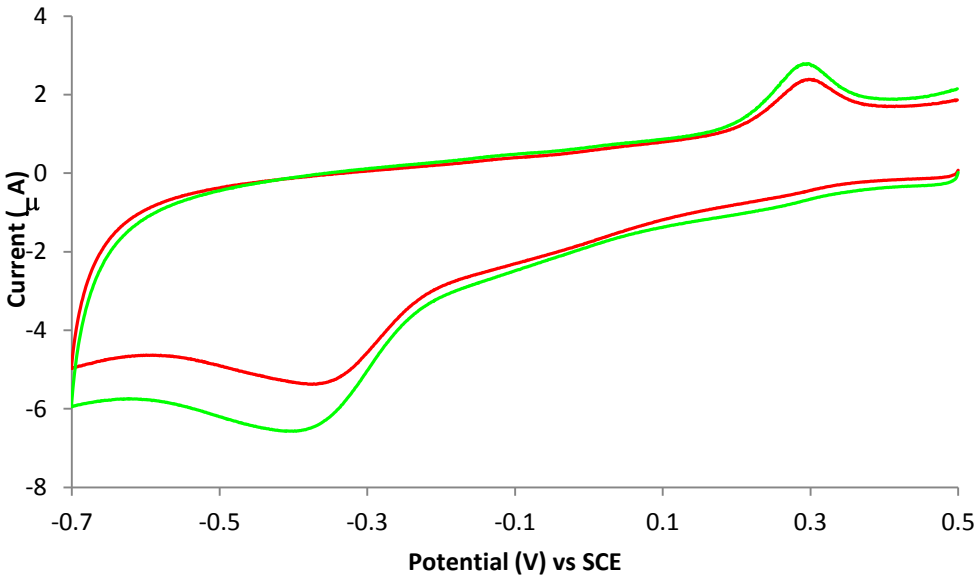


Figure 4.6. CVs showing first scan of sticky surface modified with grafting condition V in 0.1 M H_2SO_4; subjected to 1 hour of immersion in 0.1 M H_2SO_4 (red line) and sodium citrate (green line).

Figure 4.6 shows the CVs of sticky surface grafted with condition V subjected to one hour immersion in 0.1 M H_2SO_4 (red line), and sodium citrate (pH ~ 5.8) solution (green line). The broad diazonium reduction peak starting at ~0.4 V has disappeared and the peak areas at $E_{p,c} \approx$ -0.35 V are smaller (particularly after immersion in 0.1 M H_2SO_4)

compared to that of the film scanned immediately after formation (Figure 4.1). These results suggest that the sticky surface is not stable under those conditions. This is in contrast to the sticky surface formed on gold surfaces by Viel and co-workers.[4] Spectroscopic monitoring showed the film to be stable for up to two hours in air. However, the stability of a film in solution and air is expected to be different. Further investigations into the stability of the sticky surface by reaction with thiol-capped Au-np are discussed in Chapter 5.

4.3.1.3. Oxidation of Sticky Surface

Investigation into the yield of conversion of AP groups into diazonium functionalities in the films was conducted by monitoring the AP groups remaining after reaction of the AP film with $NaNO_2$. The oxidative current more positive of 0.8 V in the CV (Figure 4.7, black line) clearly shows that not all of the amine functionalities have been converted to the diazonium functionalities. (Note, this film had been reduced prior to the oxidation scans.) Based on XPS data, Gooding and co-workers[2] estimated that the percentage conversion of AP to diazonium functionalities in their films was 10 %. Comparing the CV (black line) in Figure 4.7 with the CV of an AP film formed under the same conditions (Figure 3.4, black line), it is clear that significantly more than 10 % of amine groups have reacted under the present conditions. It is possible that azo groups contribute to the XPS signal assigned to amine groups by Gooding and co-workers.[2]

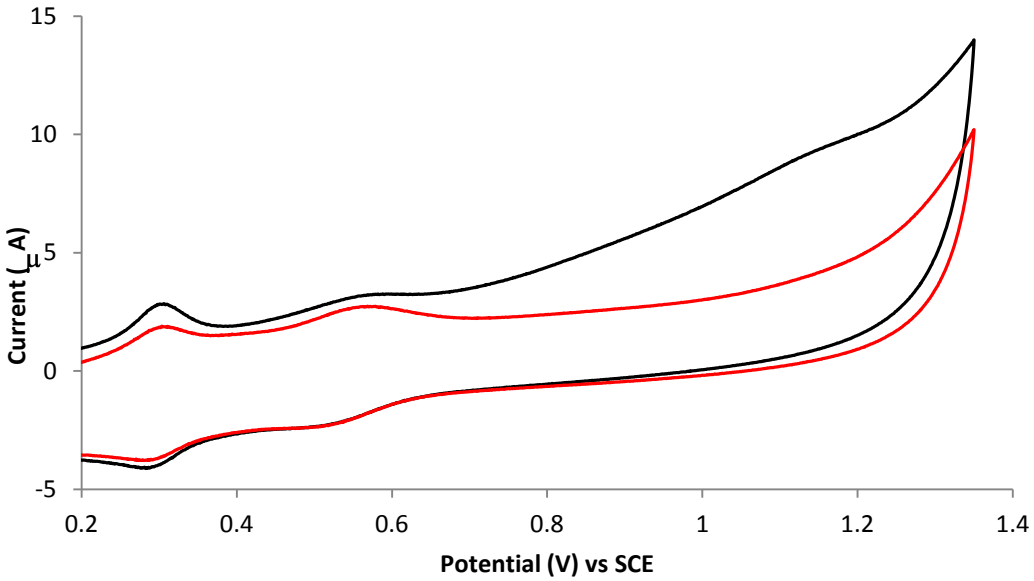


Figure 4.7. CVs of sticky surface modified with grafting condition V in 0.1 M H_2SO_4. The black line represents the 1st scan and the red line represents the 2nd scan.

4.3.2. Reaction of Sticky Surface with Au-nps

4.3.2.1. Electrochemistry of Surface bound Gold Nanoparticles

Gold is known to have a distinct electrochemical characteristic when scanned in protic solution,[22, 23] with a broad oxidation peak around +1.1 V and a sharp reduction peak at +0.8 V vs SCE (Figure 4.8). Anodic oxidation of gold surface results in the formation of $Au(OH)_3$:[24]

$$Au.H_2O + 2\ H_2O \quad \longleftrightarrow \quad (Au(OH)_3)_s + 3H^+ + 3e^-$$

The gold oxide is then reduced back to gold according to the reaction:

$$(Au(OH)_3)_s + 3e^- \quad \longrightarrow \quad Au_s + 3OH^-$$

The amount of gold oxide formed on the surface can be determined from the peak area of the gold oxide reduction. The reduction peak is used instead of the oxidation peak due to its distinct sharpness. The area under the reduction peak can be calculated using a curve

fitting program, Linkfit.[25] From the associated charges (Q_{ox}), the surface area of the gold oxide formed can be calculated according to the equation shown:

(1)

(2)

The charge associated with the reduction of gold oxide at surface (Q_{ox}) is determined by the area under reduction peak divided by the scan rate (ν). The surface area of gold oxide formed (SA_{ox}) is obtained by Q_{ox} divided by the charge required to reduce a monolayer of gold oxide ($Q_{monolayer}$ = 0.386 mC cm^{-2}).[26]

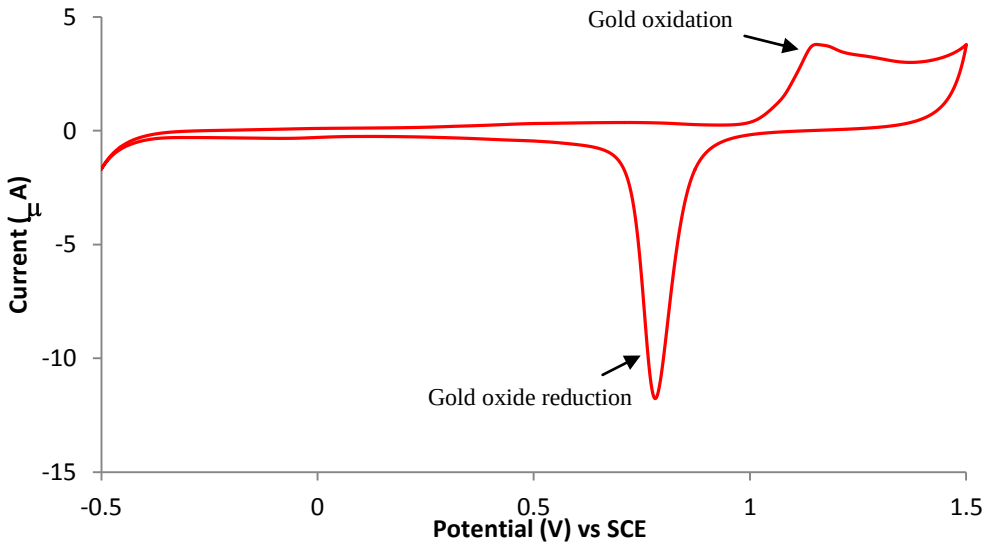


Figure 4.8. CV of a polycrystalline gold disk electrode in 0.1 M H_2SO_4.

Au-nps assembled on surfaces have similar electrochemical characteristics to bulk gold (Figure 4.9). From the equations 1 and 2 above, A_{ox} is proportional to Q_{ox} which in turn is proportional to the area under the reduction peak. The scan rate used is the same for all of the experiments with Au-nps in this work, and $Q_{monolayer}$ is constant. Therefore, the size of

the gold oxide reduction peak is sufficient to give a visual indication of the relative amounts of Au-nps assembled on different surfaces.

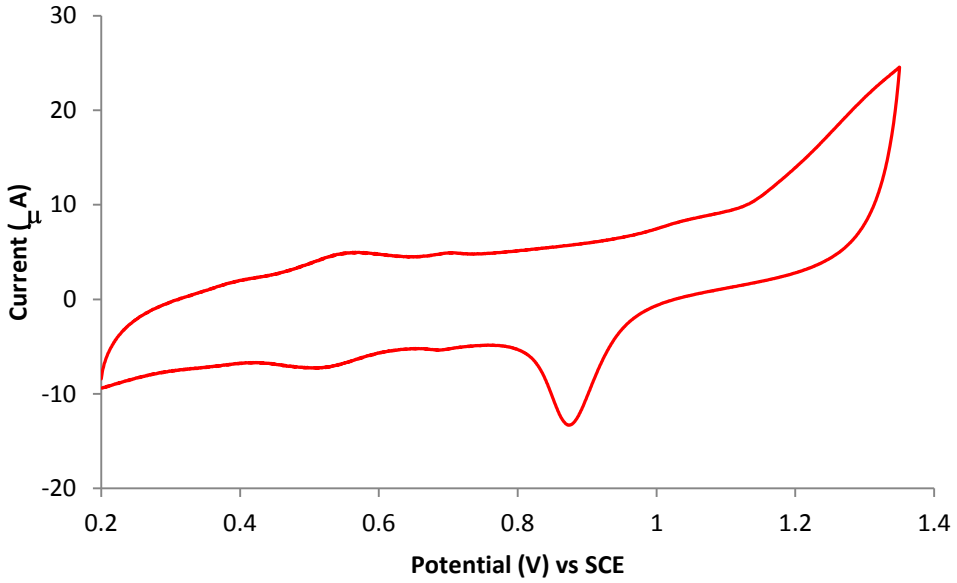


Figure 4.9. CVs of Au-nps assembled on thin AP modified GC by immersion in Au-np solution for 1 hour. Scans were carried out in 0.1 M H_2SO_4 at a scan rate of 100 mV s^{-1}.

The outcome of the cyclic voltammetric analysis of gold can be affected by several factors, such as electrolyte identity, concentration and pH, temperature and polarization time.[26] Therefore, it is important to establish the maximum response for the cyclic voltammetric analysis of Au-nps attached to modified GC. Figure 4.10 shows a plot of gold oxide reduction peak area against the applied potential (the oxidation potential was applied for 10 s) for the formation of gold oxide. For these experiments and all others involving citrate-capped Au-np in this chapter, a film with grafting condition II (Section 2.6.1) was used, unless stated otherwise. The gold oxide reduction peak increases as the applied oxidation potential increases until it reaches a maximum at an oxidation potential of +1.35 V after which it decreases as the Au-nps start to dissolve. The behaviour is the same for Au-nps assembled on both AP films and the sticky surface. Therefore, in this work the upper limit chosen to scan for Au-oxidation and reduction peak is 1.35 V with a 10 s holding time.

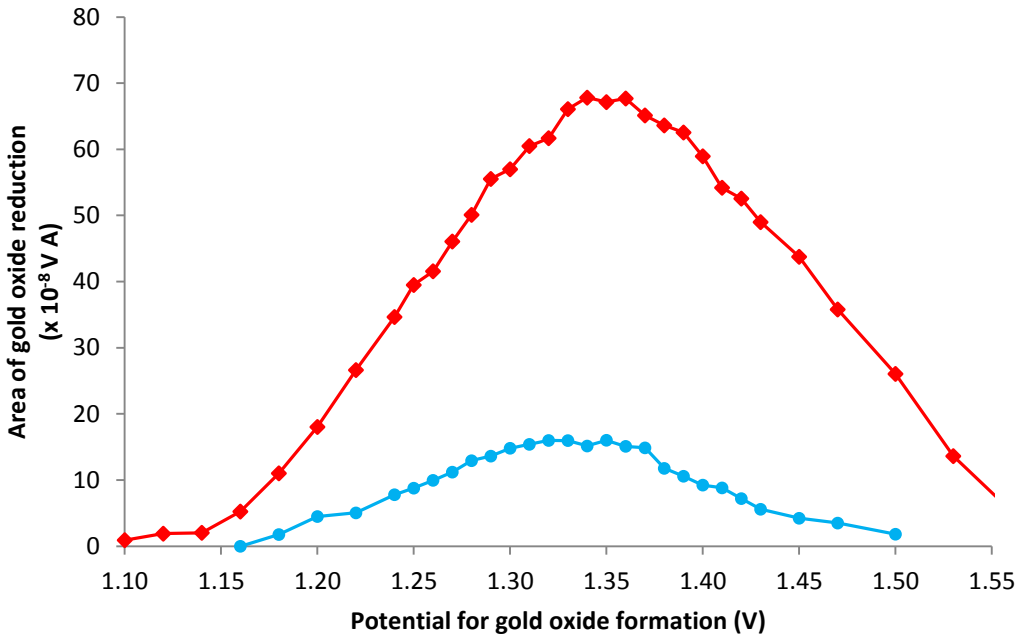


Figure 4.10. A plot of Au-np reduction peak area against the applied oxidation potential for Au-nps assembled on AP modified GC (red coloured) and sticky surface (blue coloured) by immersion in Au-np solution for 1 hour. Gold oxide reduction peak was obtained from CV scans in 0.1 M H_2SO_4 from 0.2 to the upper limit, at 100 mV s^{-1} with a holding time of 10 s at the upper limit. Peak area was calculated using Linkfit software.

4.3.2.2. Reaction of AP Film with Citrate-Capped Au-np

Before examining the interaction of citrate-capped Au-nps with the sticky surface, the assembly of citrate-capped Au-nps at AP films was characterised. Citrate-capped Au-nps are generally assumed to assemble on amine modified GC surfaces via electrostatic interactions,[27, 28] although, some workers have proposed that aromatic amines are bound to gold to form a covalent N−Au bond.[2] In this work, negative charged citrate-capped Au-nps are used, and the Au-np solution has pH of 5.8. Aniline has a protonation constant of 4.6 however the protonation constant of the AP film is probably higher. CV studies of redox probes shown in Figure 3.5 (Chapter 3) are consistent with the AP film having a nett positive charge at pH 7, and the extent of protonation will be greater at pH 5.8. This means that it is likely that the citrate-capped Au-nps assemble on the AP modified film both through electrostatic interactions between the negative charged Au-nps and

positively charged film, and also by formation of covalent bond between the NH_2 and the Au.

The stability of citrate-capped Au-np assemblies on AP film was examined by sonication in NaOH and incubation in thiol solution. Figure 4.11 shows CVs of a citrate-capped Au-np assembly on an AP film before (black line) and after (red line) sonication in 0.1 M NaOH for 1 minute. The strongly basic solution is expected to deprotonate any ammonium groups in the AP film, removing electrostatic attractions between the film and the citrate-capped Au-nps and thus decreasing the number of Au-nps assembled on the film. Clearly there is no significant change in the electroactive surface area of gold on the surface after treatment in NaOH, indicating no change to the Au-np assembly. This result is different to that reported by Downard and co-workers,[28] who found that on an aliphatic amine film, citrate-capped Au-nps aggregated, when sonicated in NaOH solution, decreasing the apparent surface area of gold. Hence, electrostatic interactions are assumed to be not important at the AP film.

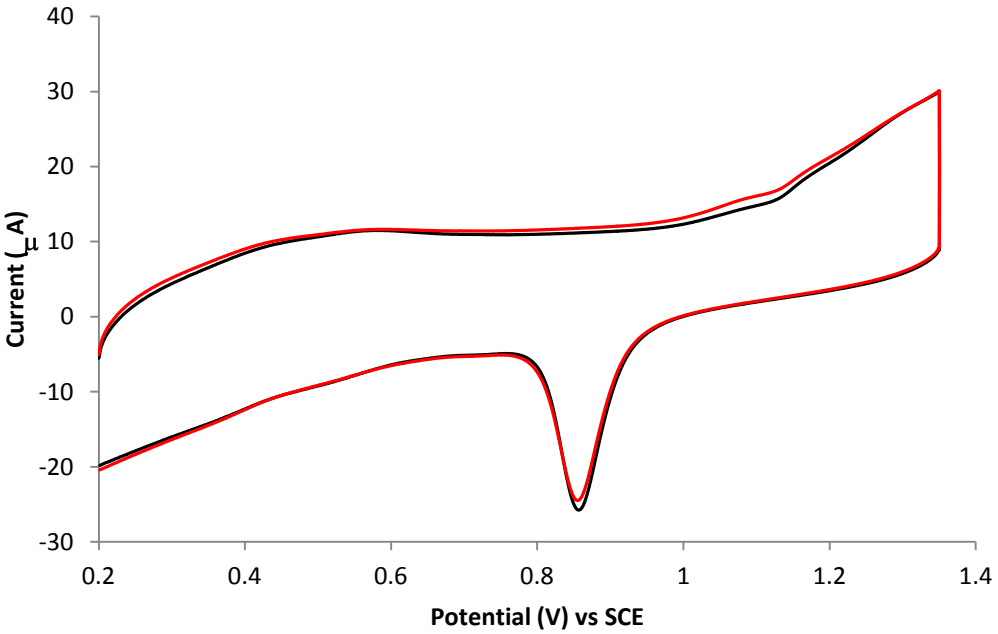


Figure 4.11. CVs of Au-nps assembled on AP film by immersion in Au-nps solution for 1 hour: before sonication (black line) and after sonication (red line) in 0.1 M NaOH for 1 minute. Scans were carried out in 0.1 M H_2SO_4.

A second method of probing the stability of the citrate-capped Au-np assembly was to treat the assembly with a thiol solution. According to a paper by Willner and co-workers,[29] thiol functionalities will replace citrate around the Au-np to produce an uncharged thiol-capped Au-np. Amine–gold bonds are known to be weaker than thiol–gold bonds,[30] therefore the displacement of an amine–gold bond to form a thiol–gold bond is expected. Leff and co-workers[31] have suggested that the bond between the gold and amine is a weak covalent bond. Figure 4.12 shows SEM images of citrate-capped Au-np assembled on AP modified GC surface before (a) and after (b) immersion in 1-decanethiol in ethanol solution. After thiol immersion the Au-nps have aggregated to form islands of Au-nps confirming that the thiol has replaced the amine N allowing the Au-nps to become mobile on the surface.

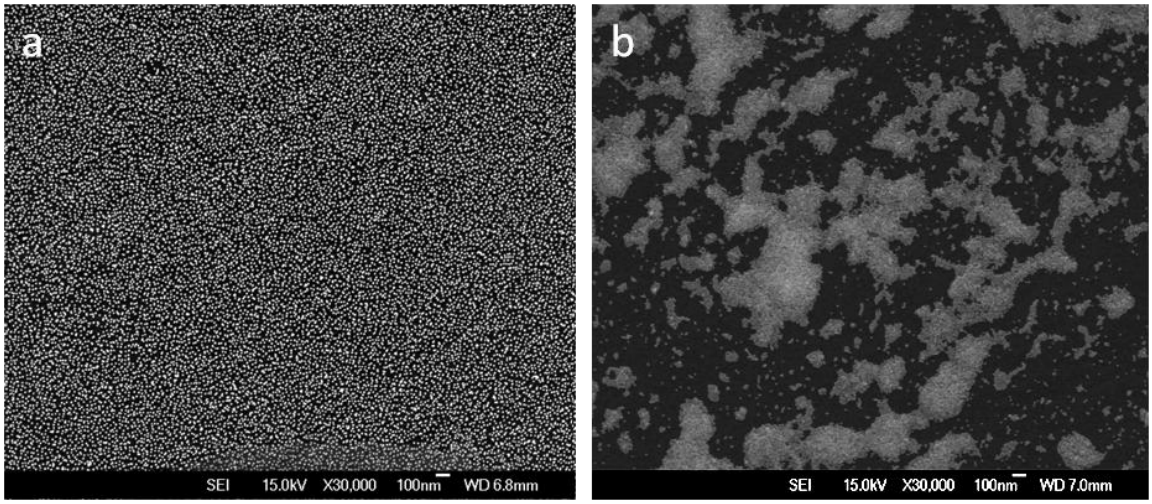


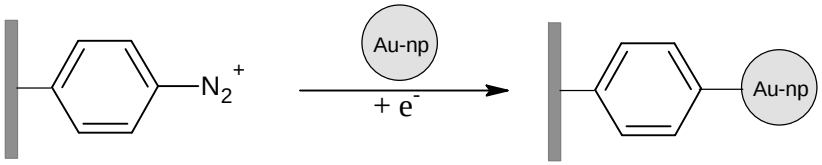
Figure 4.12. SEM images of Au-nps assembled on GC modified with AP film by immersion in Au-np solution for 1 hour (a), and then immersion in 1-decanethiol/EtOH for 24 hours (b).

4.3.2.3. Reaction of Sticky Surface with Citrate-Capped Au-np

Au-nps attached onto surfaces via both amine and thiol linkages have relatively low stability,[32, 33] therefore it is desirable to design a more stable Au-np modified surface. One method of achieving this is to use diazonium cation chemistry by forming a carbon–gold

bond.[33-36] Aromatic amine–Au, aromatic thiol–Au and aromatic carbon–Au bonds have bond strengths of 58 kJ mol^{-1}, 119 kJ mol^{-1} and 133 kJ mol^{-1}, respectively.[34, 37]

During the course of this thesis work, a paper has been published by Gooding and co-workers,[2] which describes the attachment of 27 nm diameter citrate-capped Au-nps onto a sticky surface by electrochemical initiation. The proposed reaction scheme is shown in Scheme 4.4 where the electron is provided by the electrode. Electrochemical reduction of sticky surface in the presence of Au-nps converts the diazonium functionalities to radicals (with the loss of N_2) that react with the Au-nps leading to the formation of covalent C–Au bonds.[2] Gooding and co-workers[2] showed that the attachment of citrate-capped Au-nps onto sticky surface is stable under sonication in MQ water and electrochemical treatment in dilute sulphuric acid. Similar conditions were therefore used to investigate the sticky surface in this work.



Scheme 4.4. Reaction of sticky surface with Au-nps to form covalent Au–C bond.

Figure 4.13 shows the CVs of citrate-capped Au-nps (26 nm diameter) assembled on AP films (black line), sticky surface before sonication (red line), sticky surface after 5 minutes sonication in MQ water (green line) and sticky surface after 10 minutes sonication in MQ water (blue line), where reaction with the sticky surface was electrochemically initiated. All of these films were grafted using the method of Gooding and co-workers[2] as described in Section 2.6.3. For all of these films, the surface was placed in citrate-capped Au-np (26 nm) solution and two CV cycles were performed between 0.6 and -0.8 V at 100 mV s^{-1}. After rinsing, the surfaces were placed in 0.1 M H_2SO_4 solution for analysis of the gold oxide reduction peak. The attachment of citrate-capped Au-nps (26 nm) to the AP film under the short time interval (i.e. the time taken

for two cycles between 0.6 to -0.8 V at 100 mV s^{-1}), is not possible (black line). This results agrees with that of Gooding and co-workers.[2] Figure 4.13 (red line) shows the CV of citrate-capped Au-np (26 nm) attachment to sticky surface under electrochemical conditions. These CVs suggest that the sticky surface reacts with Au-nps, via the loss of N_2, by forming a Au–C covalent bond rather than via the amine functionalities of the unconverted AP groups. However, upon sonication in MQ water for 5 minutes, the gold oxide reduction peak reduced significantly (green line) and a further 5 minutes sonication reduces the reduction peak even further. This result is in contrast to that of Gooding and co-workers,[2] who claimed that the attachment of citrate-capped Au-nps onto sticky surface is stable under extensive sonication. The reason for the discrepancy in these results is not known. These results obtained in this thesis work do not provide convincing evidence that Au-nps assemble on the sticky surface via the pathway shown in Scheme 4.4.

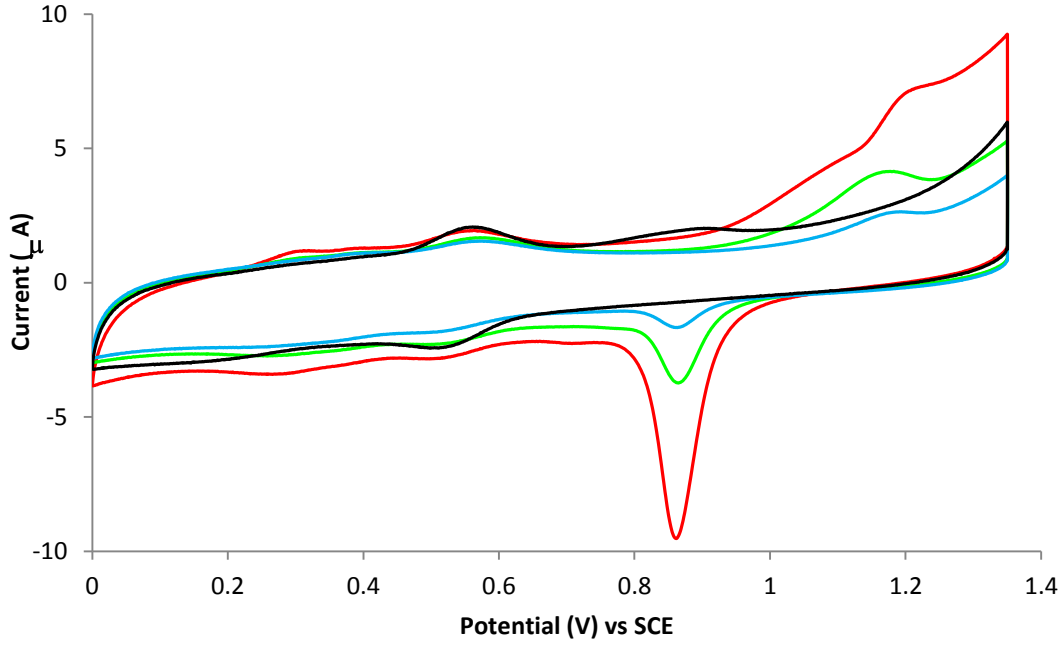


Figure 4.13. CVs of citrate-capped Au-nps (26 nm diameter) assembled on AP film (black line), sticky surface before sonication (red line), sticky surface after 5 minutes sonication in Milli-Q water (green line) and sticky surface after 10 minutes sonication in MQ water (blue line) in 0.1 M H_2SO_4. All surfaces were grafted according to Gooding and co-workers' method.[2] Citrate-capped Au-nps were assembled on the surfaces by electrochemically scanning the modified electrode in the Au-nps solution for two cycles from 0.6 to -0.8 V vs. SCE at 100 mV s^{-1}.

Spontaneous grafting of dizonium salts onto gold surfaces has been reported.[14, 15] Spontaneous reduction of diazonium salts leading to the formation of aryl layers on Au-nps has also been observed by McDermott and co-workers.[38] Therefore in principle, sticky surface may also react with Au-np spontaneously to form a covalent (C–Au) bond between the surface and the nanoparticle (Scheme 4.5, where the electron in provided by the Au-nps). Figure 4.14 shows the CVs, obtained before sonication, of citrate-capped Au-nps (17 nm diameter) attached to sticky-surface (red line) and AP film (black line) by immersion in citrate-capped Au-nps for one hour. The gold oxide reduction peak of the citrate-capped Au-nps assembled onto sticky surface is smaller than the reduction peak of Au-nps assembled on AP film.

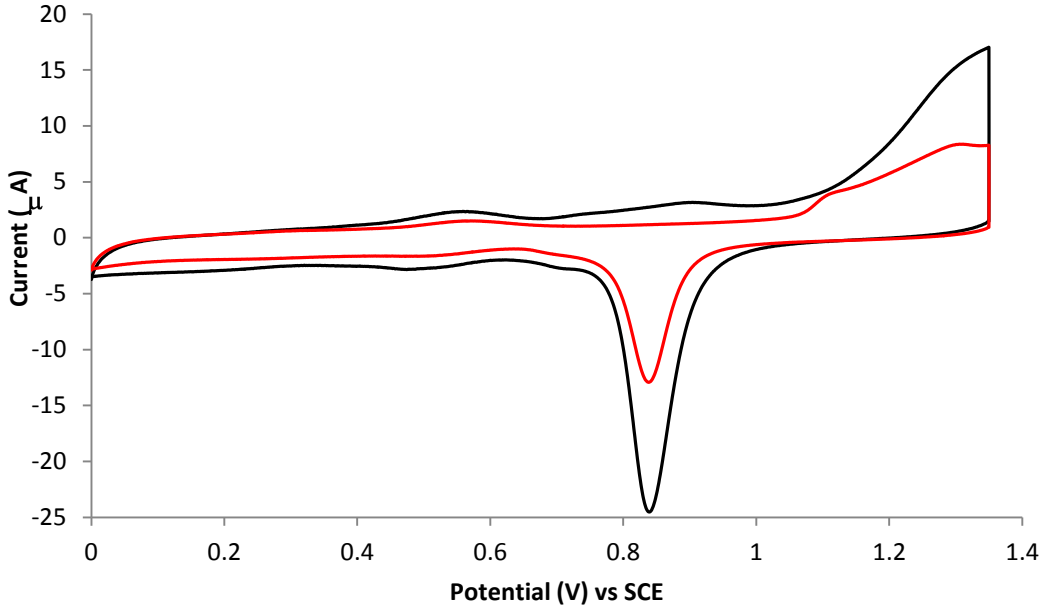


Figure 4.14. CVs of citrate-capped Au-nps (17 nm) assembled on AP film (black line), sticky surface (red line) before sonication by immersion in citrate-capped Au-nps solution for 1 hour, scans were carried out in 0.1 M H_2SO_4. All surfaces were grafted with condition V.

Due to the incomplete conversion of AP groups to diazonium functionalities during sticky surface formation (Section 4.3.1.3), the sticky surface has both diazonium and AP groups. Hence citrate-capped Au-nps may assemble onto the sticky surface via reaction with the diazonium cations forming covalent Au–C bonds, however Au-nps may also assemble by

interaction with AP groups. Moreover, because diazonium cations are positively charged, there is also a possibility that the citrate-capped Au-nps are electrostatically attached to the sticky surface rather than covalently bonded. To prevent possible reaction with AP groups, the sticky surface was oxidised before it was reacted with Au-nps, to make sure that no amine terminated functionalities were present. As discussed in Chapter 3, AP groups are irreversibly oxidised but the oxidation product is unknown. Figure 4.15 shows the CVs of citrate-capped Au-nps (17 nm) assembled on oxidised AP film (black line) and oxidised sticky surface (red line). The gold oxide reduction peak from the oxidised AP film is similar to the corresponding peak obtained from the sticky surface. Therefore, a definite conclusion about whether spontaneous reaction of Au-nps with sticky surface has occurred cannot be made. Further investigation of sticky surface with thiol-capped Au-nps is discussed in Chapter 5.

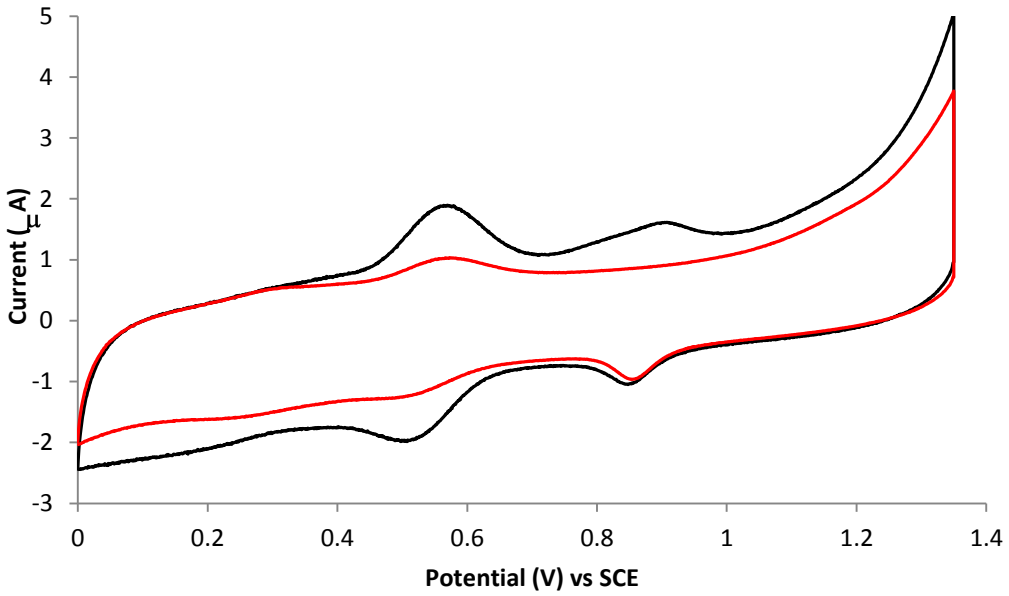
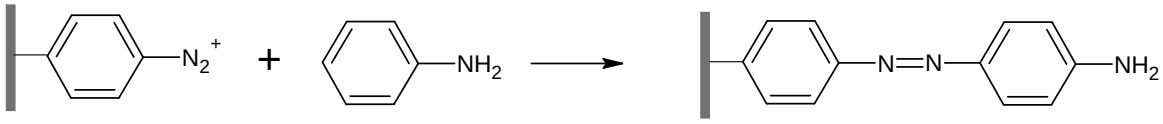


Figure 4.15. CVs, obtained before sonication, of citrate-capped Au-nps (17 nm diameter) assembled on oxidised AP film (black line), and oxidised sticky surface (red line) by immersion in citrate-capped Au-nps solution for 1 hour. Scans were carried out in 0.1 M H_2SO_4. All surfaces were grafted with condition V.

4.3.3. Reaction of Sticky Surface with Aniline

Coupling reactions of an aryldiazonium salt with an aromatic compound, such as aniline, produces an azo compound.[39] Therefore, in principle, a similar reaction should occur between sticky surface and aniline to produce a surface with amino functionalities (Scheme 4.5).



Scheme 4.5. Propose reaction between sticky surface and aniline.

The amino functionalities formed from the proposed reaction of sticky surface with aniline can be detected in protic conditions using cyclic voltammetry (Section 3.3.2.2). AP films were grafted using conditions VI and VII and converted to sticky surfaces. The sticky surfaces were immersed for 30 minutes in 1 M, and for 1 hour in 0.2 M aniline solution in acetonitrile for condition VI and VII respectively, and then sonicated as described in Section 4.2. Figure 4.16 shows the CVs of aniline attached to sticky surface (red line) and AP film (black line) in 0.1 M H_2SO_4. The sticky surface was oxidised prior to reaction with aniline to make sure no amine functionality is present before the reaction is undertaken. The presence of the larger oxidation peak on the sticky surface at $E_{p,a} \approx 0.8$ V (red line) for both grafting condition VI (Figure 4.16a) and VII (Figure 4.16b) compared to that on the oxidised AP film (black line), suggests that the reaction between the sticky surface and aniline has occurred spontaneously. The redox couple at $E_{½} \approx 0.5$ V is probably due to the formation of polyaniline on the films, during grafting of the AP films.

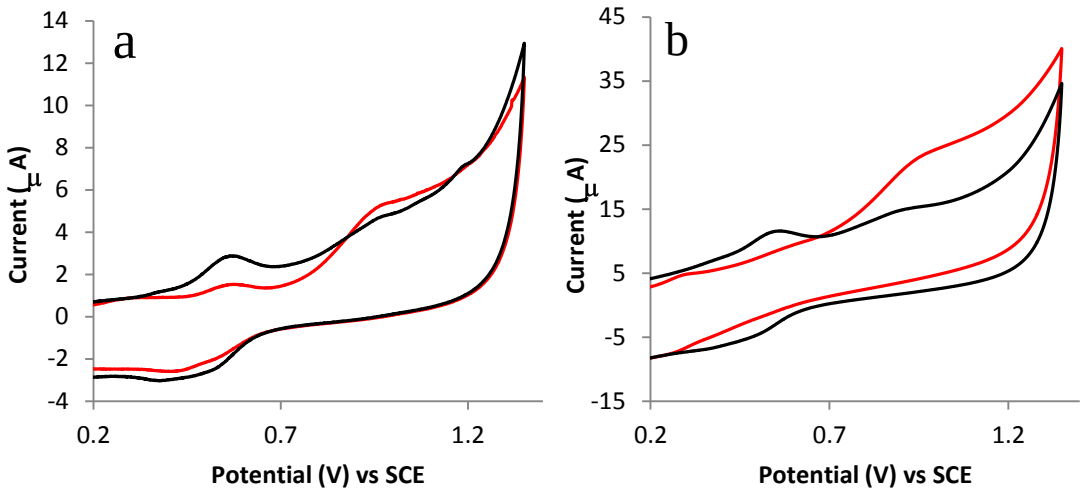


Figure 4.16. CVs showing the 1st scan of aniline attached to oxidised sticky surface (red line) and oxidised AP film (black line): a) with grafting condition VI, followed by immersion in a solution of 1 M aniline in acetonitirile solution for 30 minutes; b) with grafting condition VII, followed by immersion in a solution of 0.2 M aniline in acetonitrile solution for 1 hour. Scans were carried out in 0.1 M H_2SO_4.

4.4. Conclusions

Sticky surface was obtained by the conversion of AP films into diazonium terminated film by addition of sodium nitrite in acidic conditions. Electrochemistry was used to investigate the diazonium terminated surfaces, as diazonium moiety is an electro-active species with an irreversible redox system. The products of the electro-reduction of diazonium modified GC surface are not known. Other redox processes in the diazonium terminated films were tentatively assigned to azo/hydrazobenzene redox reactions. The CVs of the amine oxidation of sticky surfaces suggest that a low concentration of AP moieties remain in the film after the film is reacted to give diazonium functionalities.

Electrochemically initiated reactions of sticky surface with citrate-capped Au-nps shows that Au-nps are assembled on the surface via the diazonium terminated functionalities. However, the attachment of Au-nps to the surface was not as stable as suggested by Gooding and co-workers,[2] and the attachment may be either electrostatic or covalent (or a

mixture of both). Spontaneous reaction of sticky surface with citrate-capped Au-nps was attempted to investigate the reactivity of Au-nps towards diazonium functionalities. Sticky surface has fewer citrate-capped Au-nps assembled on the surface compared to AP modified surface. The positively charged diazonium ions on the sticky surface may allow electrostatic attachment of the negatively charged citrate-capped Au-nps. Therefore, a definite conclusion on covalent grafting of Au-nps on GC surface spontaneously via sticky surface cannot be made. To examine this issue further, investigation into the reaction of the sticky surface with uncharged thiol-capped Au-nps is discussed in the following chapter.

Reaction of sticky surface with aniline carried out spontaneously showed that azo coupling reaction had occurred between the diazonium functionalities of the sticky surface and the (presumably) *para* carbon of the aniline giving a surface bearing amine functionalities that can be detected electrochemically.

4.5. References

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Chapter 5. Immobilisation of Gold Nanoparticles on Glassy Carbon electrodes via Sticky Surface

5.1. Introduction

Gold nanoparticles (Au-nps) can have excellent electrocatalytic properties compared to bulk gold due to their large surface area to volume ratios.[1, 2] Au-nps has been shown to catalyse the oxidation and reduction of hydrogen peroxide (H_2O_2),[3, 4] oxidation of ascorbic acid and dopamine,[5-7] oxidation of nitric oxide (NO),[8] oxidation of carbon monoxide (CO),[9] oxidation of methanol[10] and reduction of oxygen.[11-13] Moreover, selective detection of dopamine and uric acid in the presence of ascorbic acid has also been achieved at Au-np electrodes.[5, 6]

Several strategies have been used to immobilise Au-nps on electrode surfaces including immobilisation through covalent or electrostatic interactions with the self-assembled monolayers (SAMs),[5, 14, 15] immobilisation through electrostatic interactions of amine terminated surface and citrate-capped Au-nps,[4, 16] and also covalent immobilisation through a diazonium cation terminated surface.[15] In this Chapter, the immobilisation of Au-nps is achieved by spontaneous reaction of thiol-capped Au-nps with sticky surface. The reactions of thiol-capped Au-nps with sticky surface are used to investigate the stability of the diazonium cations of the sticky surface. Finally, the electrocatalytic properties of the immobilised Au-nps towards the oxidation of ascorbic acid are discussed.

5.2. Experimental

Sticky surfaces were prepared from grafting condition V according to the procedures described in section 2.5.1 and 2.5.2, unless specified otherwise. This is referred to as the standard method. Spontaneous reaction of sticky surface with thiol-capped Au-nps was carried out by immersion of the sticky surface in thiol-capped Au-np solution for 1 hour. The thiol-capped Au-np solution was prepared as described in section 2.2.2.2 and was used as prepared. After modification with the thiol-capped Au-np, the surfaces were washed with toluene, acetonitrile and subjected to sonication in MQ water and toluene for five minutes each and dried under a stream of nitrogen, unless specified otherwise.

All electrochemical experiments at GC were conducted using GC disk electrodes (area = 0.071 cm^2) as the working electrode, Pt mesh as auxiliary electrode and SCE as reference electrode. The polycrystalline Au working electrode (area = 0.008 cm^2) was prepared before use as follows: it was polished with 1 micron alumina, sonicated in MQ water for 5 minutes and potential cycled in aqueous 0.01 M $HClO_4$ solution between 0 to 1.45 V at 50 mV s^{-1} until the CV showed no changes between consecutive cycles. The electrochemistry of GC modified with thiol-capped Au-nps was investigated in 0.1 M H_2SO_4. All CVs were recorded at a scan rate of 100 mV s^{-1}, unless specified otherwise. The electro-oxidation of ascorbic acid was carried out in 0.2 M PB (section 2.2.1) containing 1 mM ascorbic acid. All experiments were carried out at room temperature, unless stated otherwise.

5.3. Results and Discussion

5.3.1. Reaction of Sticky Surface with Thiol-Capped Au-nps

As discussed in Chapter 4, when citrate-capped Au-nps assemble on a sticky surface, it is not possible to confidently state that there is a covalent bond between the Au-nps and the film. It is possible that Au-nps assemble due to electrostatic forces between the positively charged diazonium moieties and the negatively charged Au-nps. Therefore, to further investigate the interaction of Au-nps with the sticky surface, uncharged thiol-capped Au-nps were prepared and reacted with the film. Au-nps capped with decane thiols were synthesised by the two-phase system described in section 2.2.2.2 (Chapter 2). The Au-nps were characterised by TEM and were shown to have diameters of < 3 nm (Figure 2.1, Chapter 2). Use of Au-nps with a neutral capping group avoids the possibility of electrostatic attraction to the sticky surface.

Two AP films were grafted to GC electrodes using condition V (section 2.5.1) and one of the films was converted to a sticky surface. The surfaces were immersed in thiol-capped Au-np solution for 1 hour. After rinsing with toluene, acetonitrile and water, the surfaces were placed in 0.1 M H_2SO_4 solution for analysis of the gold oxide reduction peak. Figure 5.1 shows the CVs of thiol-capped Au-nps assembled on AP film (black line) and the sticky surface (red line). For the AP film (black line), there is a very small gold oxide reduction peak indicating that a low concentration of Au-nps assemble on the AP film. Thiol-capped Au-nps are stabilised by a hydrophobic C_{10} alkyl chain (Section 2.2.2.2), and therefore may assemble on the aryl film via hydrophobic interactions. This is assumed to account for the small amount of Au-np assembly on the AP film. However, the gold oxide reduction peak from the AP film is insignificant compared to the corresponding peak obtained from the sticky surface (red line). These CVs suggest that

the sticky surface reacts with Au-nps by an additional mechanism, presumably via the loss of N_2, by forming a C–Au covalent bond. However, sonication of the sticky surface with assembled Au-nps in MQ water for 5 minutes (green line), decreased the size of the gold oxide reduction peak significantly, while further sonication in toluene for 5 minutes (blue line) only slightly decreased the gold oxide reduction peak. These results suggest that the first sonication process removes the weakly bound Au-nps (physisorbed via hydrophobic interactions) and during the second sonication the covalently attached Au-nps are stable and remain on the surface. This experiment provides the first firm evidence that Au-nps can be covalently attached via the sticky surface. Furthermore, it is clear that the Au-nps react spontaneously with sticky surface and no electrochemical assistance is required.

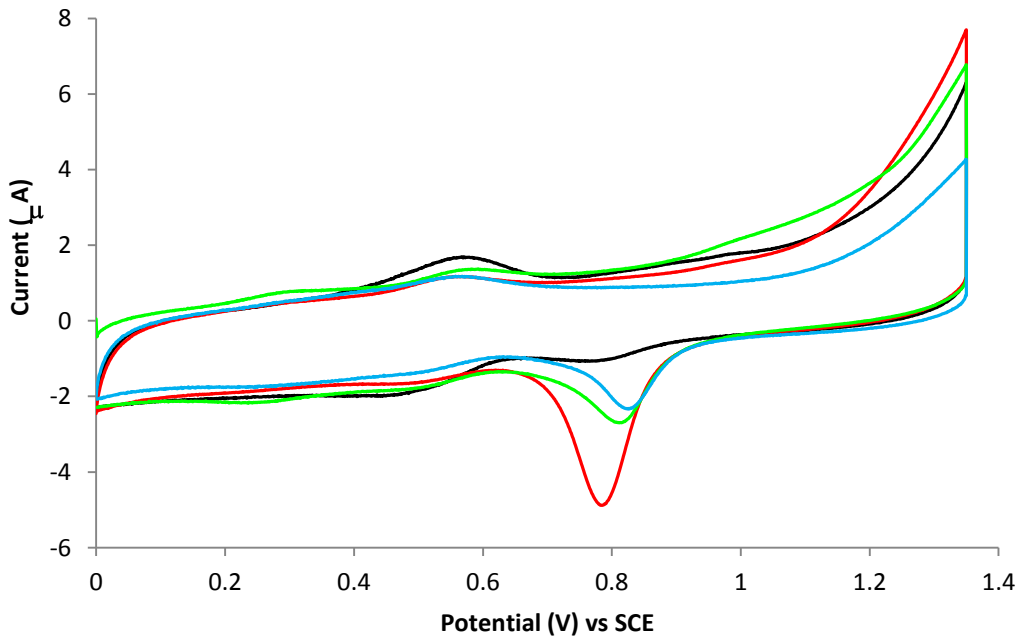

Figure 5.1. CVs, obtained in 0.1 M H_2SO_4, of thiol-capped Au-nps assembled on AP film before sonication (black line), sticky surface before sonication (red line), sticky surface after 5 minutes sonication in MQ water (green line) and sticky surface after 5 minutes sonication in toluene (blue line). All surfaces were grafted with grafting condition V. Thiol-capped Au-nps were assembled on the surfaces by immersion in the Au-nps solution for 1 hour.

As mentioned in Chapter 4, aryldiazonium cations are generally unstable and spontaneous reduction of aryldiazonium cations in solution by adventitious impurities or by the GC

substrate is possible.[17] Therefore, further investigation into the stability of sticky surface was undertaken using the reaction with thiol-capped Au-nps as the model system. For these investigations, it was assumed that the size of the gold oxide reduction peak is proportional to the number of thiol-capped Au-nps on the surface, and that (after sonication) these Au-nps are covalently attached via reaction with diazonium functionalities.

To test the stability of the sticky surface over time, sticky surface was immersed in 0.1 M H_2SO_4 for 1 hour and then immersed in thiol-capped Au-nps for 1 hour. After this procedure, the electrode was sonicated in MQ water and toluene for 5 minutes each, dried under a stream of N_2, and the gold oxide reduction was recorded in 0.1 M H_2SO_4 (Figure 5.2, red line). The gold oxide reduction peak is much smaller than that recorded at a surface which had not been immersed in H_2SO_4 for 1 hour (Figure 5.2, black line), suggesting that a much smaller number of Au-nps attach to the sticky surface that was treated in H_2SO_4 for 1 hour. Hence, it is concluded that the diazonium cations on the sticky surfaces are not stable when the film is immersed in H_2SO_4 for 1 hour. This result is in agreement with the results on Chapter 4, where the broad diazonium reduction peak started at ~0.4 V disappeared after film treatment in 0.1 M H_2SO_4 for 1 hour (Figure 4.6, red line).

Another set of experiments was undertaken to examine the short-term stability of the diazonium cation-terminated film. It was considered that during the conversion of AP groups to diazonium moieties, newly formed diazonium groups could be reduced by electron transfer from the GC substrate. Such a reaction would be consistent with the solution based spontaneous reduction of aryldiazonium salts by GC substrates.[17] To examine this possibility, a potential of 0.5 V was applied to the GC during the conversion of AP films to diazonium functionalities in the $HCl/NaNO_2$ medium. Immediately after

this reaction, the modified GC was washed with MQ water, dried under a stream of N_2, and immersed in thiol-capped Au-nps for 1 hour. After the immersion, the modified electrode was sonicated in MQ water and toluene for 5 minutes each, dried under a stream of N_2 and the gold oxide reduction was recorded in 0.1 M H_2SO_4 (Figure 5.2, green line). The gold oxide reduction peak for this surface (green line) is smaller than the corresponding peak formed by attachment of Au-nps via the standard method of forming sticky surface (black line). These CVs show that applying a positive potential during the formation of sticky surface (to prevent any reduction of the diazonium cations by the GC surface) does not increase the number of Au-nps attached to the surface, but in fact decreases the number of Au-nps. A tentative explanation for this surprising result is that applying a potential during the conversion of AP films to diazonium functionalities may affect the rate of conversion of AP groups to diazonium functionalities. A positive surface charge at 0.5 V might be expected to decrease the rate of formation of diazonium cations. Any effect on the possible subsequent reduction of diazonium groups by the substrate cannot be detected under these conditions. Therefore, these results cannot be used as evidence that diazonium cations on the surface are unstable due to reduction by the GC substrate.

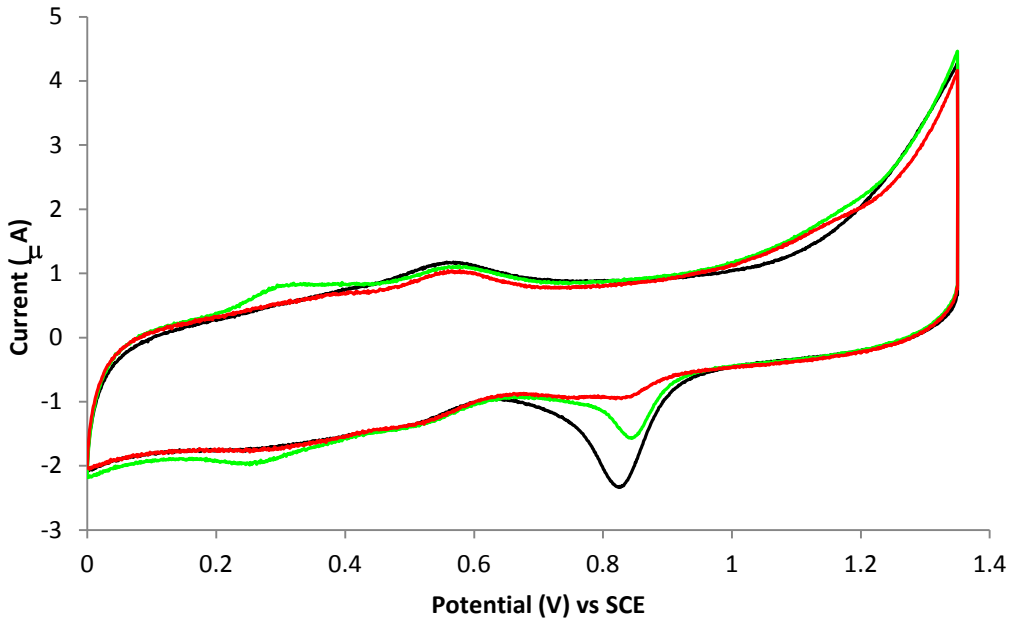

Figure 5.2. CVs, obtained in 0.1 M H_2SO_4, of thiol-capped Au-nps assembled on sticky surface. **Black line**: sticky surface was formed using the standard method and immersed in thiol-capped Au-nps solution for 1 hour immediately after formation. **Red line**: sticky surface was formed using the standard method and immersion in 0.1 M H_2SO_4 for 1 hour before immersion in thiol-capped Au-nps solution for 1 hour. **Green line**: sticky surface was formed by applying a potential of 0.5 V for 5 minutes in $NaNO_2$/HCl and immersed in thiol-capped Au-nps solution for 1 hour immediately after formation. All surfaces were grafted with grafting condition V. All surfaces were sonicated as described earlier.

Diazonium salts are more stable at low temperature and diazotisation in homogeneous solution is usually carried out at 0 ^oC.[18] Therefore, to further investigate the stability of the sticky surface, the conversion of AP films into diazonium functionalities was carried out at low temperature. GC electrode modified with AP film was immersed for 30 minutes in a solution of $NaNO_2$/HCl in an ice bath. A longer reaction time than the standard time was used to account for the lower rate of reaction at low temperature. Immediately after the modification, the electrode was washed with MQ water, dried under a stream of N_2, and immersed in thiol-capped Au-np solution at room temperature for 1 hour. After the immersion in Au-nps, the modified GC was sonicated in MQ water and toluene for 5 minutes each, dried with a stream of N_2 and the gold oxide reduction was

recorded in 0.1 M H_2SO_4 (Figure 5.3, red line). The CVs in Figure 5.3 show that the gold oxide reduction peak for the sticky surface formed at low temperature (red line) and room temperature (black line) are very similar. This indicates that either sticky surfaces are not unstable on the short time scale or that some diazonium functionalities are rapidly decomposed even at low temperature.

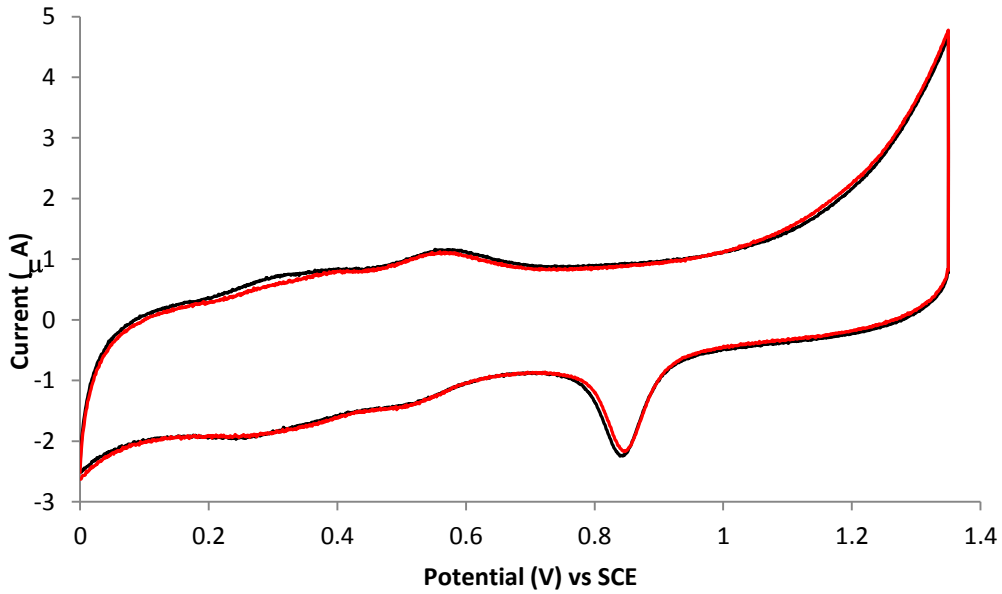

Figure 5.3. CVs, obtained in 0.1 M H_2SO_4, of thiol-capped Au-nps assembled on sticky surface. Sticky surface was formed by immersion of the AP modified GC in $NaNO_2$/HCl for 30 minutes at room temperature (black line) or in an ice bath (red line). Thiol-capped Au-nps were assembled on the surface by immersion in the Au-nps solution for 1 hour. All surfaces were grafted with grafting condition V.

Evidence presented in Chapter 4 suggested that diazonium functionalities in the sticky surface were reduced in the very broad cathodic process starting at ~0.4 V (Figure 4.1). In a final set of experiments using the assembly of thiol-capped Au-nps as a model system, the question of the reduction potential of the diazonium groups of the sticky surface (Chapter 4) was revisited. Thiol-capped Au-nps were reacted with sticky surfaces after the surfaces had been reduced at various potentials. (Reduction decomposes the diazonium cation to unknown product(s).) Electrochemically reducing the sticky surface will decrease the number of diazonium cations on the surface, which in turn decreases the

number of Au-nps attached to the surface and thus decreases the size of the gold oxide reduction peak.

Four freshly prepared sticky surfaces were reduced electrochemically in 0.1 M H_2SO_4 by performing two CV cycles from 0.5 V to negative potential limits of 0.15, 0, -0.2, or -0.6 V at a scan rate of 100 mV s^{-1}. The potential was held at the negative limit for the amount of time required to give a total reduction time of 104 s. For example, two CV cycles between 0.5 and -0.6 V at a scan rate of 100 mV s^{-1} take 44 s, so the potential was held at -0.6 V for a further 60 s to give a total time of 104 s. Similarly, for the cycles between 0.5 and 0.15 V, the potential was held at 0.15 V for 90 s. After the reduction scans, the modified GC electrode was washed with MQ water, dried with a stream of N_2 and immersed in thiol-capped Au-nps for 1 hour. After the Au-nps immersion, the modified GC was sonicated in MQ water and toluene for 5 minutes each, and dried with a stream of N_2, the gold oxide reduction was recorded in 0.1 M H_2SO_4 (Figure 5.4). As a control sample, a fifth sticky surface was immersed in 0.1 M H_2SO_4 for 104 s (no potential was applied) before immersion in thiol-capped Au-nps (Figure 5.4, black line).

As shown in Figure 5.4, reducing the sticky surface at 0.15 or 0 V results in a similar size gold oxide reduction peak (orange and blue line respectively). These peaks are smaller than the gold oxide reduction peak from the control sample (black line), providing indirect evidence that a significant proportion (approximately 30%) of diazonium cations in the films are reduced at these potentials. When a more negative potential of -0.2 V was applied to the sticky surface (green line), the gold oxide reduction peak is very small and after reduction at -0.6 V (red line), the gold oxide reduction peak is barely visible, indicating that only a very small number of Au-nps are attached to the surface. These CVs confirm the findings of Chapter 4: the diazonium cations in the film reduce over a broad potential range from 0.2 V (or higher) to -0.6 V. It is likely that the diazonium cations in

different film environments reduce at a different potentials, giving this broad reduction manifold. As noted earlier, different film environments arise due to the different environment of amine groups in the AP film (Chapter 3).

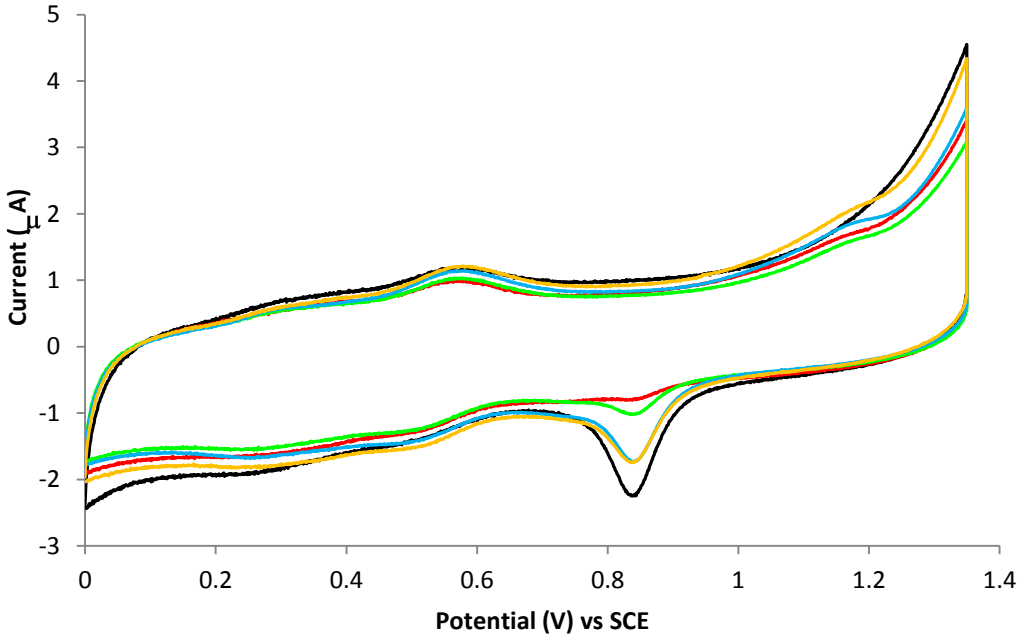

Figure 5.4. CVs, obtained in 0.1 M H_2SO_4, of thiol-capped Au-nps assembled on (electrochemically reduced) sticky surface. Sticky surface was electrochemically reduced in 0.1 M H_2SO_4 by scanning two CV cycles from 0.5 V to a negative potential limits of: 0.15 V (**orange line**), 0 V (**blue line**), -0.2 V (**green line**), and -0.6 V (**red line**) at a scan rate of 100 mV s^{-1}. The poetential was held at the negative limit for the amount of time required to give a total reduction time of 104 s. The **black line** represents the control sample (i.e. sticky surface was immersed in 0.1 M H_2SO_4 for 104 s without applied potential.) Sticky surface was formed using the standard method. Thiol-capped Au-nps were assembled on the surface by immersion of the reduced sticky surface in the Au-nps solution for 1 hour. All surfaces were grafted with grafting condition V.

5.3.2. Oxidation of Ascorbic Acid

Ascorbic acid is found in many fruits and is an essential compound for metabolic reaction in animals and plants. The concentration of ascorbic acid in biological fluids can be used as a marker for the oxidative stress of human metabolism.[19] Excessive oxidative stress has been linked to cancer, diabetes and hepatic disease.[19, 20] Therefore, it is of interest to be able to design a probe to determine the concentration of ascorbic acid with high

reproducibility. The formal potential for oxidation of ascorbic acid is -0.187 V (vs SCE) at pH 7,[21] however the oxidation process is typically slow at solid electrodes.[5, 6, 21]

Au-nps have been shown to have a catalytic effect towards the oxidation of ascorbic acid.[5] For example, citrate-capped Au-nps assembled on a thiol monolayer modified Au electrode, decrease the overpotential for the oxidation process by about 210 mV compared to a bulk gold electrode.[5] In this section thiol-capped Au-nps attached to the GC electrode via the sticky surface (as discussed above) were briefly tested for their electrocatalytic activity towards the oxidation of ascorbic acid. Figure 5.5 shows the CVs obtained at a bare Au electrode in a solution of 1 mM ascorbic acid in 0.2 M PB solution. The shape of the large peak at low potential, $E_{p,a} \approx 0.02$ V, on first scan (black line) and behaviour on the subsequent scans suggest that the peak corresponds to oxidation of adsorbed ascorbic acid on the electrode, whereas, the broad low peak at $E_{p,a} \approx 0.39$ V is assigned to the oxidation of solution-based ascorbic acid. On the other hand, on bare GC (Figure 5.6) there is only a peak arising from the adsorption of ascorbic acid on the electrode and no clear peak from the oxidation of solution-based ascorbic acid.

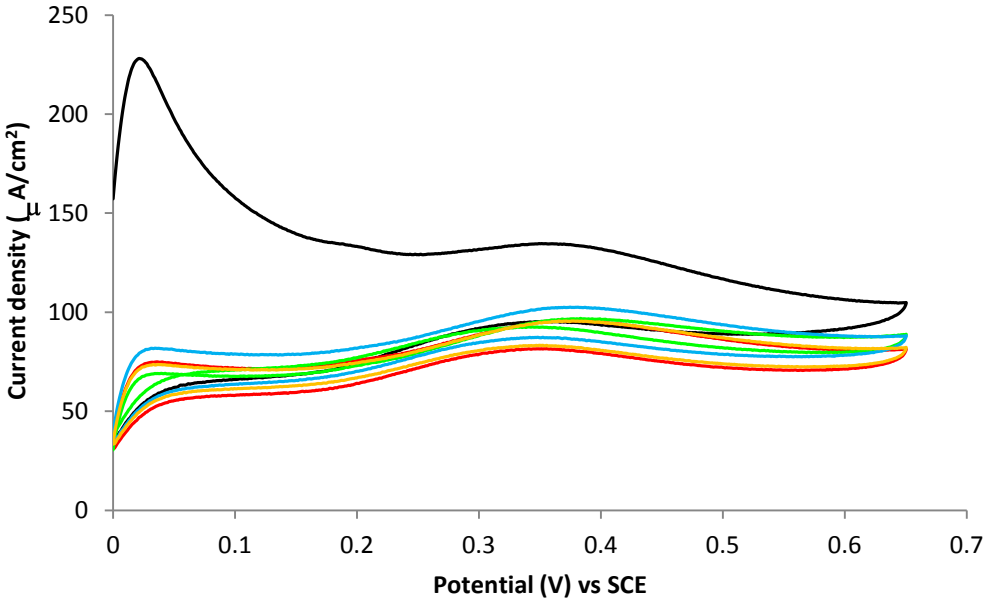

Figure 5.5. CVs of bare Au electrode in 0.2 M PB solution containing 1 mM of ascorbic acid at a scan rate of 50 mV s^{-1}. The black line represents the first scan, and the subsequent scans are represented by red, green, blue, and orange lines respectively.

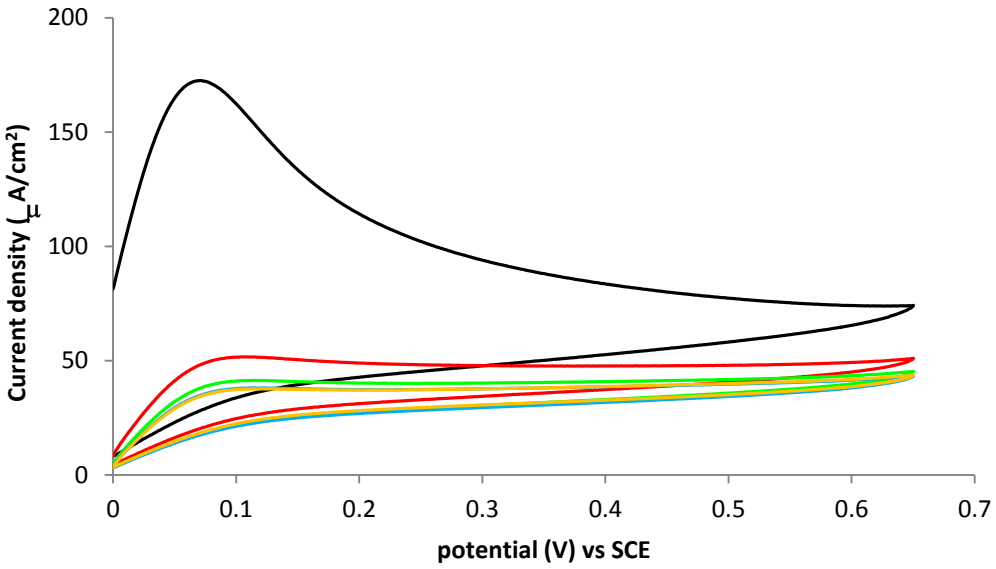

Figure 5.6. CVs of bare GC electrode in 0.2 M PB solution containing 1 mM of ascorbic acid at a scan rate of 50 mV s^{-1}. The black line represents the first scan, and the subsequent scans are represented by red, green, blue, and orange lines respectively.

The oxidation of ascorbic acid at AP modified GC (Figure 5.7) occurs between 0.3 to 0.4 V and there is no visible peak at $E_{p,a} \approx 0.02$ V. The existence of the film prevents adsorption of ascorbic acid on the electrode. On all of these surfaces, the second and subsequent scans have relatively low current densities (current was normalised by dividing the current by the geometric electrode area) compared to that at the Au-np modified GC electrode (Figure 5.8). As shown in Figure 5.8 for the Au-nps attached to GC via sticky surface, the oxidation of ascorbic acid occurs at 0.29 V, which is 100 mV less positive than the oxidation of solution based ascorbic acid at bare Au electrode (or AP modified GC). Importantly, Figure 5.8 also shows that repeat scans (from 2 to 5) give reproducible oxidation peaks for ascorbic acid with high current density.

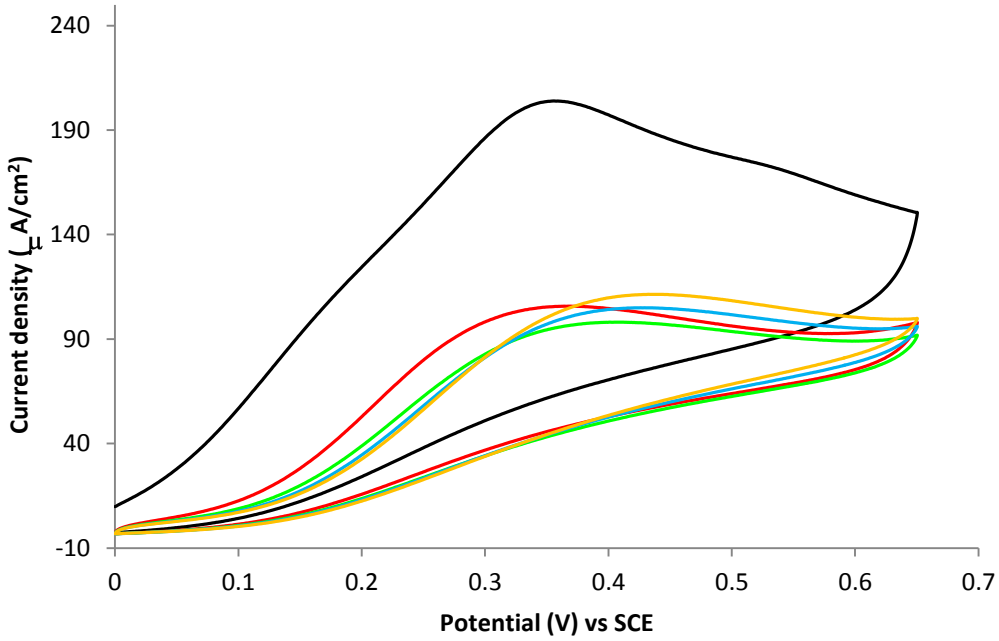

Figure 5.7. CVs of AP modified GC electrode in 0.2 M PB solution containing 1 mM of ascorbic acid at a scan rate of 50 mV s^{-1}. The black line represents the first scan, and the subsequent scans are represented by red, green, blue, and orange lines respectively. The AP modified GC electrode was carried out by grafting condition V.

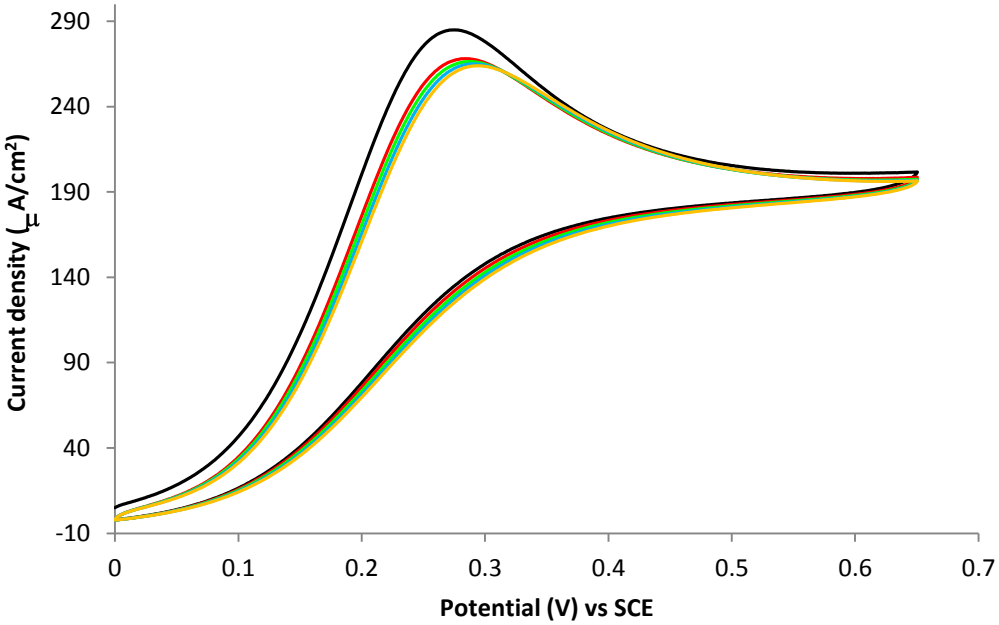

Figure 5.8. CVs obtained in 0.2 M PB solution containing 1 mM ascorbic acid of Au-nps modified GC electrode at a scan rate of 50 mV s^{-1}. The black line represents the first scan, and the subsequent scans are represented by red, green, blue, and orange lines respectively. The Au-nps was assembled on the GC electrode via sticky surface.

The gold oxide reduction peaks for Au-nps modified GC (Figure 5.9, red line) are significantly smaller than that of the bare Au electrode (black line). Comparing the active surface area of the Au-nps and bulk gold (0.64 and 1.95 mm^2 respectively) indicates that the Au-nps have a total surface area of 33% of the bulk gold electrode. (Surface area was calculated using the equations 1 and 2 shown in Chapter 4, and by assuming the charge to reduce a monolayer of gold oxide is 0.386 mC cm^{-2}.[22]) Figures 5.5 and 5.8 show that on repeat scans, the oxidation current density for ascorbic acid is approximately 2.6 times greater at Au-np modified GC than at the bulk Au electrode. (Note that the current density for the Au-np electrode in Figure 5.8 is based on geometric area of the GC electrode.) After adjusting for the actual surface area of gold, these results indicate an overall 71-fold greater sensitivity for oxidation of ascorbic acid at the Au-np modified GC electrode than at bulk gold. It is clear that the Au-nps have significant electrocatalytic activity towards the oxidation of ascorbic acid.

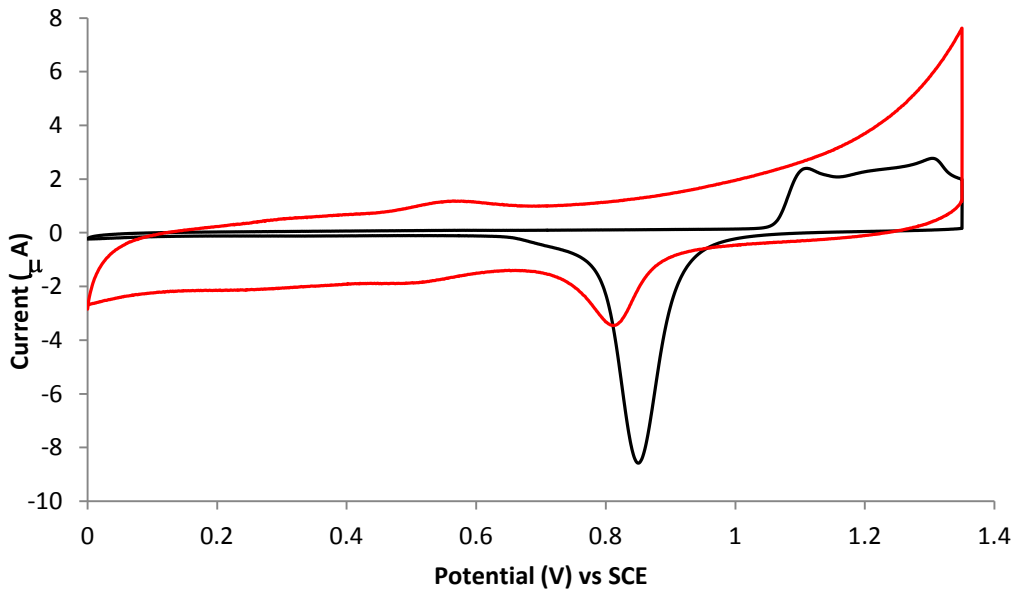

Figure 5.9. CVs obtained in 0.1 M H_2SO_4 of bare Au electrode (black line) and Au-np modified GC electrode (red line).

5.4. Conclusions

The spontaneous reaction of sticky surface with thiol-capped Au-nps was investigated electrochemically in 0.1 M H_2SO_4 by analysing the Au-nps gold oxide reduction peak. The CVs suggest that the sticky surfaces react with the Au-nps, via the loss of N_2, by forming covalent Au–C bonds. It was shown that the Au-nps react with the sticky surface spontaneously and no electrochemical assistance was required. The reaction of sticky surface with thiol-capped Au-nps was also used as a model system to study the stability of the diazonium cations terminated sticky surface. It was confirmed that sticky surfaces are not stable in acidic condition. The instability of the sticky surface maybe caused by the reduction of the diazonium cations by the GC electrode or via decomposition by adventitious impurities. Further studies need to be carried out to investigate the stability of the diazonium cations terminated sticky surface.

The reduction potential of sticky surface was also investigated using the reaction of the electrochemically reduced sticky surface with thiol-capped Au-nps. It was found that the diazonium cations of the sticky surface reduces at a broad potential from 0.4 V to -0.6 V, which is in agreement with the findings in Chapter 4. This conclusion is based on the assumption that the number of Au-nps on the surfaces (obtained from the gold oxide reduction peak) is a direct representation of the number of diazonium cations on the sticky surface.

GC electrode modified with thiol-capped Au-nps shows electrocatalytic activity towards the oxidation of ascorbic acid. The ascorbic acid oxidised at a less positive potential at the Au-nps modified electrode compared to the bulk Au electrode. Moreover, the oxidation peak current density is also greater at the Au-nps modified GC electrode compared to the

bulk Au electrode. Further studies are needed to optimise the catalytic activity of Au-nps and explore their activity for other substrates.

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Chapter 6. Conclusion and Future Work

The ability to covalently assemble a target species onto a substrate is important for applications such as, sensors, catalysis and molecular electronics. One of the possibilities to achieve covalent bonding is to utilise aryldiazonium chemistry. The concept of a diazonium cation terminated film which could react with many solution species giving a direct covalent bond to the surface is very attractive. Accordingly, the aim of this thesis work was to investigate the formation of diazonium cation terminated surface (sticky surface). Gold nanoparticles (Au-nps) were chosen as a target species to investigate the reactivity of the sticky surface. The successfully attached Au-nps were also investigated for their catalytic activity towards ascorbic acid.

Sticky surface was obtained from the reaction of aminophenyl (AP) film with $NaNO_2$/HCl. Therefore, it was of interest to investigate the AP modified GC electrode. The characterisation of AP groups was carried out electrochemically by investigating the oxidation of the AP groups in protic aqueous conditions. In addition, ferricyanide and ruthenium hexammine redox probes were also used to investigate the AP modified surface before and after electro-oxidation in protic aqueous conditions. Before electro-oxidation process, the surface has a nett positive charge, which was converted to a neutral or negatively charged surface upon electro-oxidation in the protic aqueous conditions. The electrochemistry of AP film in acidic aqueous solution shows that there are a number of oxidisable species over a broad potential range. The product(s) of the electro-oxidation of the AP films was not investigated in this thesis.

Sticky surface was characterised electrochemically by investigating the reduction of the diazonium cation in protic aqueous conditions. The reduction of the diazonium cations

appears to occur over a broad potential range from 0.4 to -0.6 V. The electrochemical study of the sticky surface suggests that the reaction of AP films with $NaNO_2$/HCl gives rise not only to diazonium cations but also to other unidentified products. The unknown product(s) and the product(s) of the electro-reduction of the diazonium cations of the sticky surface were not studied further.

The reactivity of the sticky surface was investigated by reacting the sticky surface with citrate-capped Au-nps. It is unclear whether the citrate-capped Au-np actually reacts with the sticky surface giving a covalent bond between the film and the nanoparticle, or alternatively, whether the negatively charged Au-np is electrostatically assembled on the positively charged sticky surface. Moreover, the number of Au-nps that assemble on the sticky surface is less than those that assemble on the corresponding AP film, and the Au-nps assembly on the sticky surface is less stable than that on the AP film.

To address the complication due to the possibility of electrostatic interactions between the sticky surface and the Au-nps, non-charged thiol-capped Au-nps were employed to investigate reaction at the sticky surface. Spontaneous assembly of thiol-capped Au-nps on sticky surface has been achieved, and comparison with control experiments suggests that attachment of the thiol-capped Au-nps is via the formation of covalent bonds. However, it is unclear whether the reaction occurs due to the spontaneous reduction of the diazonium cations by the electrode, or due to the reduction of the diazonium cations of the sticky surface by the Au-nps. Both pathways are expected to give a film incorporating aryl radicals which then react with the Au-nps to form a covalent Au–C bond.

The reaction of thiol-capped Au-nps was further used as a model system to investigate the stability of the sticky surface and the possibility of spontaneous reduction of the diazonium cations by the underlying GC substrate. It was found that the sticky surface is

not stable over a one hour period in acidic solution. Electrochemically holding the AP modified GC at a positive potential during the sticky surface formation in the $NaNO_2$/HCl solution (to prevent the reduction by the GC) did not increase the number of Au-nps assembled on the surface. However, applying a potential may change the electronic properties of the system, which may affect the formation of the sticky surface. Therefore, other approaches need to be used to address the possible spontaneous reduction of the sticky surface by the substrate.

Thiol-capped Au-nps assembled on the GC electrode via sticky surface show elecro-catalytic activity towards the oxidation of ascorbic acid. The electrochemical oxidation of ascorbic acid catalysed by Au-nps has an overpotenital 100 mV less positive than that at a bulk gold or AP modified GC electrode. In addition, higher oxidation peak current and much greater stability of the signal on repeat scans, were also observed on the Au-np compared to the bulk gold or AP modified GC electrode.

From these results, it seems that there are many uncertainties surrounding the sticky surface and further investigations need to be carried out before use of sticky surfaces can be optimised. For example, the instability of the sticky surface can be further studied by using a substrate that is known not to spontaneously reduce diazonium cations (such as a non-conductive substrate) or by forming a very thick film so that electron transfer to outer diazonium groups is not possible. These studies should clarify whether film-based diazonium cations can be spontaneously reduced by electrode materials. Reaction with thiol-capped Au-nps could be used as the test reaction for these studies.

The characterisation of the sticky surface by spectroscopic and microscopy methods such as XPS (X-ray photoelectron spectroscopy), AFM, IR and Raman could be employed to

examine the film structure. Raman spectroscopy and XPS may allow observation of a covalent Au–C bond between the surface and the Au-nps.

Reactions of sticky surface with target species other than Au-nps should also be investigated. Correct choice of the target species is important for method development, so that a clear understanding of the interactions with the sticky surface can be gained. As found in this work, uncharged species are clearly preferable to anionic species. Reactions of cationic species could also be explored. Optimisation of the amount of target species that can be attached on the electrode surface via the diazonoium cations of the sticky surface is a requirement for a practically useful modification method. Increasing the conversion of AP to diazonium groups and the stability of diazonium groups are important for this goal.